

What future for nuclear power?

The outlook for civil nuclear power has blackened everywhere in the aftermath of the Chernobyl accident. Here is what governments other than the Soviet should now do.

WHATEVER went wrong at Chernobyl, that accident has not done much for the job prospects of nuclear engineers outside the Soviet Union. To be sure, there may be a little short-term employment to be had in Austria, where the government decided last week that it will now dismantle the country's only nuclear power station, which has been waiting on the brink of full operation for several years. The Swedish government, which had already decided to build no more reactors, and to close down its nuclear industry when the dozen reactors now operating reach the end of their useful lives in 2010, now says that it may bring forward that deadline. Even in France, Western Europe's most bullish nuclear operator, discontent with the nuclear policy the new government has inherited is being demonstrated in the usual way, with huge crowds gathering around the perimeter of some nuclear plants. At least until the Soviet report of the Chernobyl accident is published, the operators of nuclear plants in Western Europe and elsewhere will find themselves on tenterhooks.

In its broadest sense, the question whether communities should make electricity from uranium is political. Voters have a right to decide whether they wish their governments to sponsor the generation of electricity by means that can cause social disruption and damage on the scale on which it has been caused in the Ukraine. Electorates have a right to know, for example, whether their governments have arrangements that will allow communities to survive serious nuclear accidents without bringing civic administrations to the point of collapse. But in at least three Western European countries, nuclear safety is bound to be an issue in general elections in the coming months. In Britain and West Germany, where pro-nuclear governments must go to the polls quite soon (Britain within two years, West Germany next spring), doubts on nuclear safety will be exploited by the opposition parties. In the Netherlands, where there are elections this week, the government has thought it prudent to side-step the issue by saying that it will not now plan to add to the two reactors now operating, as had been intended in the 1990s. In the United States, it is inevitable that Chernobyl will reinforce the opposition to plans for nuclear construction; the chance must have markedly diminished that the Long Island Lighting Company will be given a licence to operate the Shoreham plant, which is ready to operate but which has been held up for lack of a convincing plan to evacuate the locality in case of a nuclear accident. What should public utilities and their often timorous governments do now?

Decisions

The first maxim is an old one: do not panic. There is no need, for there is no evidence that nuclear accidents are catching. Probably the opposite is the truth. An accident on the scale of that in the Ukraine will, if anything, make reactor operators elsewhere even more vigilant. Quick decisions are usually costly decisions. The decision of the government of the Netherlands to scrap plans for nuclear development on the eve of this week's elections may be electorally prudent, but may cost Dutch taxpayers money in the coming decade and will not contribute one jot to the safety of the two reactors now operating. And the same is

even more true elsewhere. Many communities (in France and Japan, for example) could not turn their backs on nuclear power without giving up some of their present use of electricity. Even those inclined to follow such a course must pay at least as much attention to the safe operation of existing plants during the remainder of their operating lives.

Utilities, governments and their taxpayers should also remember that nothing has happened in the past three weeks to diminish the economic prize to be won by the safe operation of nuclear energy. In very round numbers, a kilogram of uranium can now be bought on the open market for \$100, but will yield thermal energy worth \$1,000 if used as fuel in a thermal reactor. In a fast reactor designed for breeding fissile material, the value of the thermal energy from a kilogram of uranium may be a further 1,000 times as much. The endless argument about the economics of nuclear power turns on the question of how much of this huge economic rent must be spent on building reactors, on making them safe and on disposing of the radioactive waste materials. Even though uranium would not be as cheap if its exploitation in nuclear energy were more fashionable, the sheer size of the economic benefit cannot be ignored even now. If a country such as Britain were to replace its present annual consumption of 100 million tonnes of oil by the equivalent in uranium, the economic gain would far exceed the government's annual borrowing requirement. It would be foolhardy to ignore the size of this potential economic benefit simply because a reactor in the Ukraine went out of control last month.

Plans

Equally, however, it would be wrong for governments elsewhere to pretend that what happened at Chernobyl is irrelevant to their domestic power policies. In the litany of the hazards of civil nuclear power, the possibility of a serious reactor accident has always been the most awesome. Quite apart from the tragic loss of life in and around the Chernobyl reactor, and the social and economic disruption that the accident has caused, the consequences for the communal psychology of the region around Kiev can only be guessed at. It is natural that people elsewhere should now say that they would pay quite a high price to avoid such accidents on their doorsteps. Prudent governments will pause and reassess their plans to build reactors by some process so public that their constituents will be confident in whatever decisions emerge. Access to a full explanation of what happened at Chernobyl, promised last week by Mr Mikhail Gorbachev, will be an essential starting point for that.

In the West, the process will be complicated by the degree to which civil nuclear power has already been turned into a public and political issue by a gaggle of environmental movements whose motives are rarely as innocent or even respectable as advertised. The West German Greens, for example, likely to emerge from next year's election in a government coalition with the Social Democrats, are as fierce about the supposed evils of the corporatist state as about those of nuclear power. In countries such as Britain, the bubbling opposition to the search, by the public agency responsible for radioactive waste disposal (Nirex), for sites at which to bury low-level waste springs not



from a realistic assessment of the hazards but from the more familiar reluctance of those who enjoy the benefits of modern industry to put up with inconvenient side-effects. Deciding on rational policies for nuclear energy will require that those with an inner certainty that nuclear energy is an abomination should be restored to the pathways of rationality. The gravity of the accident at Chernobyl may, paradoxically, help by showing level-headed people that there are serious arguments on both sides of the question.

Research

Most governments will also recognize that it is a misfortune that so many of them have deliberately allowed research and development programmes in nuclear energy to shrink. In the design of thermal nuclear reactors for example, there has been hardly any research that matters for close on a quarter of a century, since most governments and their construction companies took the view (early in the 1960s) that they knew enough about the subject to make a go of it. The result is that the technology of nuclear power has been immovable for far too long, and in particular since before the potential seriousness of loss-of-coolant accidents (part of the trouble at Chernobyl) was recognized by studies carried out in the United States in the early 1970s. It is as if the technology of air transport had been fixed at the outbreak of the Second World War. Hopes by some of the laboratories with continued interest in reactor design, the Argonne National Laboratory for example, to attract funds for projects aimed at inherently safe reactor systems, have not been conspicuously successful.

Much the same is true of research on waste disposal. People either worry about the isolation from the rest of the environment of entire irradiated fuel elements or they worry about storing the mixed fission products from separation plants; in each case, there is a huge amount of radioactivity at the beginning, but it is also necessary to isolate some low-activity components (actinides) for millennia on end. Technically, the problem would be simplified if the wastes were separated chemically, but the only serious studies made of this possibility are desk studies purporting to show that such sophistication would be uneconomic on the basis of assumptions valid in the 1970s, not now when people may be prepared to spend more for safety. However they decide to generate electricity in the years ahead, governments have a responsibility to breathe new life into more imaginative research. It would be appropriate if they did so collaboratively. In Western Europe, Euratom is still looking for a function after 30 years. The International Atomic Energy Agency, which has played a valuable role in the response to Chernobyl, could become the vehicle for a research programme that would show more convincingly which are the safest ways to go.

Technical developments by themselves will not suffice. There must also be a better public understanding of the nature of the risks. So much has been plain since long before Chernobyl. But the well-meaning attempts of public authorities at general education have been ineffectual because of their failure to distinguish between enlightenment and reassurance. Reactor accidents apart, it is probable that the people most seriously affected by the routine operations of the nuclear industry in the few places at which it flourishes are the workers at nuclear plants, reactors and reprocessing plants. Radioactive waste and routine discharges from reactors (neutron irradiation of atmospheric argon produces beta-active argon-41) have much smaller effects when measured by the yardstick of the radiation dose to a population as a whole. The obvious difficulty is that public authorities cannot go about insisting that even small doses of radiation can cause trouble (delayed cancer induction, genetic defects) without unsettling those who work for them. Much easier to behave as if these risks do not exist or to pretend that they are negligible.

The truth is that (reactor accidents apart) the risks of radiation are for most members of a population small, much smaller than

the effects of cosmic rays (a comparison valid only if there is no damage threshold) but not negligible to people who have become convinced that radiation risks, however small, are important. Sadly, the acute radiation deaths at Chernobyl (11 so far) may make ordinary people more receptive to the orders of magnitude of the different risks. Certainly, governments and public authorities cannot leave the task of public education to those now most active in the field, the environmentalists whose interest is to confuse the issue (as well as to confuse civil with military nuclear power).

None of this will, for the time being, persuade ordinary people not to fear what they have now come to fear most, the risk of a reactor accident. The fact that other reactors may differ in design from that at Chernobyl will cut no ice when the world knows that most accidents in the nuclear industry have been caused not so much by errors of design as by personal folly or incompetence, for which the euphemism is "human error". There was nothing in the design of the Brown's Ferry (Tennessee) reactor from which outsiders could infer that it would be set on fire by a man looking for a gas leak with a lighted candle. The long sequence of minor discharges of radioactivity from the British separation plant at Windscale (now Sellafield) has rightly raised in the public mind the question whether people who cannot be trusted to avoid small mishaps will ensure that large mishaps never happen.

So how are the nuclear establishments to re-establish public confidence in their competence? They could help themselves substantially by paying more attention to the design of reactors and their instrumentation with the objective of making them "user friendly", not user hostile as they tend to be at present. Whatever the technical defects of the Three Mile Island reactor that went wrong in 1978, there is no doubt in retrospect that an impending accident required the operators to behave in ways that seemed in the heat of the moment to be unreasonable. It is not surprising that they chose not to follow the instruction manual. The general technical question is that of how to design complicated machines in such a way that the human beings asked to supervise their operation are able to intervene constructively. The problem is by no means novel. Aircraft designers have grappled with it for years. Is it not time that nuclear engineers took a leaf out of this now well-read book?

Ambiguity

A further difficulty arises from the ambiguity of responsibility that afflicts those who are in charge of these complicated machines. The Challenger disaster in January seems clearly to have shown that trouble would have been avoided if it had been easier for unwelcome news to reach the top management of the US National Aeronautics and Space Administration. It will almost certainly emerge that part of the trouble at Chernobyl is that the local management was reluctant to admit to Moscow (especially on the eve of the May Day celebrations) that it had a nasty accident on its hands. What these and other accidents have shown is that there is an urgent need that technical people ostensibly in charge of complicated pieces of machinery should have more freedom in deciding how they should be operated safely, and a more direct responsibility for making sure that this is done. Their situation is not very different from that of the masters of merchant ships, who are still the ultimate arbiters of, for example, whether they should put to sea in rotten weather.

These are only some of the issues that governments and their agencies must face. All of them require a degree of flexibility and a habit of frankness that comes awkwardly to public authorities everywhere. The temptation will be to funk them. But the only course that people will respect will be to face them openly, accepting the promise of nuclear power for what it is, a potential boon that will be attainable only if people at large acknowledge that it is also safe. The task is not impossible, but will require more of governments than they have recently been inclined to show in this cause. □

Chernobyl

US physician tells heroic tale of Moscow

THE medical problems of dealing with the victims of the Chernobyl accident hospitalized in Moscow have turned out to be unexpectedly difficult, according to Dr Robert Gale, the Los Angeles physician who had been working at Moscow's Hospital No. 6 until a brief respite last weekend. Dr Gale, speaking on the telephone from Los Angeles earlier this week, said that the 299 patients at the hospital appear all to have received a damaging dose of radiation while working at or near the reactor damaged on 26 April.

According to Dr Gale, typical doses exceeded 2 Gy (1Gy=100 rad) with the doses received by the most seriously injured workers exceeding 5 Gy. Although most of the radiation exposure appeared to have been caused by gamma rays, Dr Gale explained that the difficulty of treating the patients in Hospital No. 6 arose from the variety of the means by which radiation doses had accumulated. Some people had breathed substantial doses into their lungs, others had ingested radioactive materials while other cases were complicated because the radiation appeared to have come from only one side of the body.

While emphasizing that he had no information about those in or near Chernobyl at the time of the accident apart from those in hospital in Moscow, Dr Gale thought it probable that between 50,000 and 100,000 people among the local population now evacuated would have received doses of radiation or of radioactivity large enough to warrant annual examinations.

Among those acutely injured by radiation, Dr Gale explained, the most common symptoms were gastrointestinal disturbance, liver toxicity and depression of haematopoiesis (blood-cell formation). One of the unexpected difficulties of planning to treat severely injured patients by bone-marrow transplantation had been that of tissue-typing in circumstances where blood-counts were falling rapidly.

Dr Gale's presence in Moscow apparently came about as a consequence of approaches to the Soviet authorities through Dr Armand Hammer, chairman of the Occidental Petroleum Company, and Dr Vincent de Vita, director of the US National Cancer Institute. His offer of help was apparently accepted within 24 hours. He speaks highly of the informality with which he, his colleagues and their equipment were allowed into the Soviet Union. With Dr Gale are two colleagues from the University of California at Los

Angeles, Dr Paul Terasaki and Dr Richard Champlin, and Dr Yair Reissner from the Weizmann Institute in Israel.

Much of the activity in Moscow has been concerned with identifying potential donors from among those covered worldwide by the international transplant registry, which is a listing of people with known histocompatibility characteristics. He said it was impressive that it had been possible to identify donors and to deliver tissue for transplantation to Moscow in between 48 and 72 hours. Even so, he said, most of the 35 transplants performed in recent days had used marrow taken from relatives.

Some of the Moscow patients have also received transplants of fetal liver. He

takes the view that the experience gathered in the past few weeks in Moscow will be invaluable in responding to future nuclear catastrophes, accidental or otherwise, and says that he is delighted that the Soviet authorities have agreed to full publication of the data now being gathered. He says that the information becoming available will at least double the extent of the pre-existing database.

On the preparedness of his Soviet colleagues, Dr Gale says it is plain that Soviet physicians had "given thought in advance" to the problems that would be posed by a nuclear accident on the scale of Chernobyl.

He also drew attention to the way in which the nuclear accident had stretched the resources of the international transplant registry. Matching tissue for 35 patients had had to be sought in 15 different countries. The problem of dealing with a larger accident could well be beyond present resources, he said.

Tim Beardsley & Vera Rich

SDI

Academics pledge opposition

Washington

LEADERS of a drive to discourage academic scientists and engineers from participating in research on the Strategic Defense Initiative (SDI) brought their case to Washington last week. They delivered a pledge to the White House, signed by more than 3,700 science and engineering professors, not to accept or solicit SDI funds. In addition, 2,800 graduate students signed a similar pledge not to participate in SDI-related projects.

The boycott pledge began early last summer on the campuses of Cornell University and the University of Illinois at Urbana-Champaign. Advocates of the pledge argue that it is fruitless to do research on a project that is "not technically feasible".

Furthermore, they suggest that treating SDI as a partial defence rather than a total nuclear shield against Soviet attack will make SDI merely a new weapon system guaranteed to escalate the arms race. At a press conference held in Washington last week, John Kogut, a physics professor at the University of Illinois at Urbana-Champaign and one of the instigators of the pledge, emphasized that SDI would jeopardize existing arms control agreements, such as the Anti-Ballistic Missile Treaty, and make future agreements more difficult to achieve.

Another consequence of a large funding effort for SDI, according to James Melcher, director of the electromagnetic and electronic system laboratory at Massachusetts Institute of Technology (MIT), is a gradual strangling of the US economy. Melcher sees more and more physicists,

engineers and computer scientists being drawn into weapons-related research. At the same time, he fears that corporations are placing less emphasis on developing commercially viable technologies, choosing instead to take a "quick fix" from readily available defence funds.

Although spending on SDI research has increased dramatically in the three years since President Reagan announced the programme, only a small portion of that money has actually arrived at academic institutions. According to figures compiled by the Federation of American Scientists (FAS) for a forthcoming report on SDI spending, teaching institutions have received between two and four per cent of total expenditure on SDI. In 1985, for example, SDI payments to universities totalled \$49.1 million, compared with total SDI expenditures of \$1,464.8 million. The SDI Organization has provided funds to 69 US universities so far, with Utah State leading the list, having received £27.5 million since 1983. Frank Redd, associate director for Utah State's space engineering centre, says that the bulk of that money comes from existing defence contracts, and was only recently changed to come under the SDI umbrella.

Similarly, Robert Byers, a spokesman for Stanford University, the fourth leading recipient of SDI funds according to FAS figures, explains that the bulk of that money comes from a pre-existing Air Force contract for research on free-electron lasers. But support for the pledge drive was low among physicists at Stanford, only eight of twenty-one faculty members signing the pledge. **Joseph Palca**

Particle accelerators

US agency accused of bad costing

Washington

THE US Congress is likely to scrutinize more closely costs of building particle accelerators following the publication last week of a highly critical report accusing the Department of Energy (DoE) of providing only "fragmented and incomplete" cost estimates in its budget submissions. The report*, prepared by the US General Accounting Office (GAO), recommends that congressional appropriations committees direct DoE to provide full and timely cost estimates, as well as details of technical uncertainties, of current projects.

The GAO study was requested by Senator Bennett Johnston, a Louisiana Democrat on the Senate Appropriations Committee who has made no secret of his scepticism of big accelerator projects.

Prospects of an early start on the construction of the Superconducting Supercollider (SSC) have been dealt a blow by the report, which estimates the total cost of SSC to be 60 per cent higher than DoE has hitherto indicated. DoE has made a point-by-point rebuttal, but the GAO inspectors have seen fit to make only minor changes in the light of the DoE response.

The GAO report examines DoE's six largest high-energy and nuclear accelerator physics projects and finds that DoE consistently understated total and preconstruction costs in its formal budget submissions. Preconstruction research and development for these is, according to GAO, likely to amount of \$440 million; only \$88 million has so far been formally reported in budget documents.

Preconstruction work for SSC, for example, will cost \$62 million more than the \$58 million identified in budget submissions to Congress. The Tevatron I and II projects at Fermilab will, according to GAO, cost \$180 million more than the \$16 million that has been reported. Other projects likely to cost more than indicated are the Stanford Linear Collider and the Continuous Electron Beam Accelerator Facility at Newport News, Virginia, as well as the planned Brookhaven relativistic heavy-ion collider and the Los Alamos Meson Physics Facility II (LAMPF II).

The DoE response to the charges is that much accelerator research is generic in nature, and so need not be identified with a particular project until construction is approved by Congress. GAO disagrees, saying that formal "mission-need statements" should be prepared at an earlier stage than is DoE's practice. The essence of the disagreement is the uncertain definition of the start of a project.

GAO is equally critical of DoE when it comes to estimating total costs associated with a project, however. The total cost of

SSC, according to GAO, will be \$4,900 million, \$1,900 million more than DoE estimated in testimony to Congress a year ago. GAO observes that full cost estimates for this and other projects are not presented to Congress in a coherent form before projects are approved; as a result, Congress gets no clear idea of the cost of a project until it is too late. The Stanford Linear Collider and Tevatron projects are given as examples for which Congress could have been better informed. And GAO specifically criticizes DoE for not fully explaining technical uncertainties in some projects. For Tevatron I the uncertainties led to a \$71 million cost overrun.

British research

Signs of a silver lining appear

In a week in which it seemed as if the British government would relax its policy to enforce the continued contraction of universities, a committee of interested parties has produced a telling criticism of public policy on civil research. These developments coincide with a general feeling in the research community that the pressures on the British government to pursue a less rigorous policy on public spending cannot fail to benefit research.

Precisely how the universities will benefit cannot be foreseen from the events of this week. On Tuesday, however, the University Grants Committee (UGC) was due to send to all British universities a notice of payments of recurrent grants to be expected in the academic year beginning on 1 August. This is the year in which the first effects are apparent of UGC's new policy of concentrating resources on the universities with the best records in research. Although, for this year, there is a "built-in safety net" which will ensure that no university is more than 1.5 per cent worse off than the average university (in a total budget that is reduced by 2 per cent in real terms), universities destined for sharper reductions in later years are identifiable from the letters.

The government's concession, due to be announced by the Secretary of State for Education and Science, Sir Keith Joseph, on Tuesday, is that extra financial help will be provided outside the UGC budget for institutions threatened with hardship which are able to meet certain criteria, such as the demonstration of centres of excellence in fields to which insufficient attention has been paid. This broad formula is a means by which the government could avoid the prospect of particular universities being threatened with closure in advance of the next general election. It is understood that no decision

DoE protests that some estimates have been presented at individual committee hearings, and disagrees with GAO's inclusion of ancillary equipment (for example, dedicated computing facilities) in project costs. But whichever way the issue is decided, the pressures of the Gramm-Rudman deficit reduction law suggest that Congress will want to look much harder at DoE's big science proposals in future. The administration's proposed budget for high-energy and nuclear physics in 1987 is \$771 million, an increase of 17 per cent over this year's figure, but it is already clear that Congress is unlikely to countenance an increase on that scale.

Tim Beardsley

*Nuclear Science: Information on DoE accelerators should be better disclosed in the budget. General Accounting Office, April 1986.

has been made about the scale on which extra funds would be made available.

Meanwhile, criticism of government policy on civil research has come from a committee of the Advisory Board for the Research Councils (ABRC) under P. Mathias, professor of economic history at the University of Oxford, which was set up in 1984 to assess the potential importance of private funds as a means of supporting research at academic institutions and research institutes.

The committee says that in spite of the wide discussion of research policy in Britain during the past few years, it is impressed by the sharp contrast between British spending on civil science and that elsewhere. British civil science receives 1.6 per cent of gross domestic product (GDP), compared with 2.0–2.5 per cent in the United States, West Germany and France, according to the report, which notes that British spending is declining while that in other countries is growing.

The report also says that defence research and development consumes a higher proportion of total spending, and of government spending, in Britain than elsewhere, and that industrial companies in Britain account for a smaller proportion of total research spending than in other countries. The committee makes a particular point of the apparent decline of spending on research by private industry in the recession years of 1981–1983 (when the volume of research appears to have fallen by 6 per cent). It notes that the same trend appears to have continued in 1984 (for which final figures are not available) and wryly remarks that if the disinclination of British companies to spend money on research is a permanent phenomenon, this is hardly the best climate in which academic institutions might hope to raise more support from companies. □

Human genome

Department of Energy on the map

Washington

UNTIL recently, sequencing the human genome seemed an impossibly large project, almost like counting all the grains of sand on all the world's beaches. But today the Department of Energy (DoE) is giving serious consideration to a project to sequence all of the estimated 3,500 million base pairs making up the human genetic material. Initial estimates put the cost of the entire project at \$1,800–\$3,600, or about \$1 per base pair.

Early in March, DoE held a conference in Santa Fe, New Mexico, to discuss the feasibility of undertaking the sequencing project. The conference, hosted by DoE's Los Alamos National Laboratory, produced a report outlining a strategy for accomplishing the task. According to David Smith of the Office of Health and Environment Research (OHER) at DoE, the department is expected to decide in a matter of weeks how and whether to proceed with the monumental project.

DoE has historically had an interest in the human genome, and has been supporting research into genetic damage from energy sources for some time. Together with Lawrence Livermore National Laboratory, Los Alamos has been involved in the National Laboratory Gene Library Project, an effort to construct a chromosome-specific gene library. So far, the small insert library — containing fragments of 5,000–10,000 base pairs — is complete, and work on the large insert library for fragments of up to 50,000 base pairs has begun. Los Alamos has also developed flow cytometry technology capable of rapidly separating large amounts of DNA by chromosome, a capability that Los Alamos senior fellow Mark Bitensky sees as important to the success of the project.

Bitensky is coordinating Los Alamos' effort to make the gene sequence a reality. He says the project will "require many laboratories, many funding agencies, and many technologies", but he believes it can be done, and that the time is right to do it.

Although DoE may seem like an unlikely agency to handle such a project, Bitensky and OHER head Charles DeLisi feel that DoE is well placed to take a lead role. In addition to the gene library project, DoE has the computational facilities necessary for such a large-scale project. And DoE believes it has the know-how to undertake large-scale scientific projects, pointing to the DoE-run Fermi National Laboratory as an example of its expertise.

Current plans call for a decentralized programme, with one coordinating facility organizing the work of many centres around the country. The organizers hope the sequence can be a multinational

effort. Bitensky says European interest in the project is already high.

Two theoretical approaches to the sequencing project were considered at the Santa Fe meeting. One involved randomly sequencing fragments from the genome, and trying to determine the order of the fragments on a *post hoc* basis (see below). This was rejected in favour of a scheme where the genome is first sorted by chromosome, then digested with a restriction enzyme like *Sfi* I that will produce a relatively small number of relatively large fragments. The first task would be to order these large fragments by chromosome, with a small, pilot sequencing effort started while the ordering task was being completed. Having ordered fragments not only breaks the sequencing down to a size more easily managed conceptually, but will also provide a useful tool for investigating genetic diseases, Bitensky believes.

Critics of the sequencing project do not deny that the information would be useful. Rather they worry that spending so much money on such a large project may be an inefficient use of funds. George Church of the University of California at San Francisco, a participant at the conference, echoed the widely held concern that such a large project could draw money away from other research projects. Church is working on new DNA sequencing techniques, and would like to see the project proceed. Others, including Fred Blattner of the University of Wisconsin, feel that a complete sequence from a smaller organism would yield a great deal of information about cellular processes at a small fraction of the cost. Blattner is currently working on sequencing the *Escherichia coli* genome, and others are working on the yeast and nematode genomes. Another option would be to sequence just part of one human chromosome, providing a window into human genetic control without devoting so many resources to such a major project.

Still unresolved is what to use as a starting material for the sequence project. One possibility is the DNA from a hydatiform mole. Such DNA can be produced in large quantities, but may be inappropriate as it has aberrant genetic properties. A second possibility would be to use material from a variety of different sources, perhaps a different individual for each chromosome. But critics worry that any choice will necessarily exclude information on the diversity of human genetic material.

Bitensky says that one possible method for selecting the DNA to use mentioned at the Santa Fe meeting was to hold a competition among the world's men and women. The person coming up with the largest contribution to the project would have his

The numbers game

Washington

SEQUENCING the estimated 3,500 million base pairs in the human genome is a mammoth undertaking. But Fred Blattner of the University of Wisconsin came away from the Department of Energy's March meeting on the sequence project optimistic about the prospects for completing the task in a reasonable length of time.

The approach that encourages Blattner does not require ordered clones or restriction maps. Instead, sequencing starts on random pieces of DNA. Sorting out repeating sequences may not be possible using this approach, but all the unique sequences would be uniquely assembled.

To show this approach is possible, Blattner presents the following scenario: Work-stations would be set up around the country to carry out the project. Each station would consist of two technicians and four automated sequencing machines. The machines would have 40 lanes, with each lane capable of recording 750 residues. Running the work stations in two eight-hour shifts five days per week gives: $750 \text{ residues} \times 40 \text{ lanes} \times 4 \text{ machines} \times 2 \text{ shifts} \times 5 \text{ days} = 1.2 \text{ million base pairs per week, or } 60 \text{ million per year.}$

To make sure the entire genome is covered, Blattner figures it will be necessary to calculate 10 times the number of residues in it, or 35,000 million base pairs. Using this scenario, 580 work-stations could complete the job in a year. With each station costing \$500,000, the project would cost \$290 million.

Automated sequencers with such capabilities will soon be on the market, says Blattner. Although the information produced by this random sequencing approach would not be as rich as a complete ordered sequence, it would still be extremely useful. A later step could be to put all the unique segments in order.

Despite the organizational problem of coordinating the work of so many laboratories, Blattner believes it can be done.

Joseph Palca

or her DNA sequenced.

A major player in the sequencing project could be the Howard Hughes Medical Institute, which is already supporting restriction mapping work by Raymond White at the University of Utah Medical School and Frank Ruddle at Yale University. The institute's president Donald Fredrickson confirms that there is interest in the DoE project, but wonders if the time is right to begin such an endeavour.

DeLisi, described as the driving force behind DoE's efforts on this project, says nobody gave serious thought to the idea of sequencing the entire genome as little as five years ago. Now, it is technically feasible: all that is required is the will and the money.

Joseph Palca

US research costs

White House to back down?

Washington

A STUDY by the White House Science Council calling for substantially increased federal support for university-based research met a favourable reception both in the White House and on Capitol Hill last week, amid signs that the administration may be moving towards modifying highly unpopular proposed changes to indirect cost reimbursement.

Allan Bromley, vice-chairman of the science council study, met officials of the Office of Management and Budget (OMB) and said later that he was "optimistic" that new proposals on indirect costs would be forthcoming. But there was still no word from the administration on how exactly it proposed to defuse the row over OMB's proposal to fix administrative cost reimbursement for university research at 26 per cent of direct costs in the current year (the national average), with reductions in years to come. Revised proposals are expected within the next few weeks, in time for implementation at the beginning of July.

The science council study, chaired by

David Packard, is unambiguous about the need for an increase in support for university research without taking money from research elsewhere (see below). It was given an enthusiastic welcome by members of the House of Representatives' Science Policy Task Force last week, and President Reagan was said to be writing to David Packard accepting its principal recommendations.

The study proposed adopting a uniform percentage for indirect cost reimbursement as one of several measures designed in aggregate to benefit the universities. Packard has previously spoken out publicly against the OMB decision earlier this year to adopt the recommendation on indirect cost reimbursement while ignoring recommendations to reduce amortization periods for facilities and equipment. Bromley also pointedly told the task force last week that OMB's proposal was "not at all our recommendation" and that it could lead to "substantial hardship" for universities.

John McTague, acting science adviser to the President, made clear his personal

support for the panel's report last week, telling a press conference that he would expect an increase in federal support for university research of a few tens of per cent in the President's proposed budget for 1988. And McTague reminded reporters that President Reagan, presenting awards during National Science Week last week, had said "...we understand the need to increase our investment in basic research".

McTague himself will not, however, be in the administration to see the completed 1988 budget: he is to leave the White House shortly to become chief of research at the Ford Motor Company. **Tim Beardsley**

Strident report

Washington

THE White House panel's report, released in draft form on 17 January but published formally last week, is strident in tone.

Universities now represent only \$8,000 million of the \$100,000 million in total national research and development, and the proportion is declining. After noting that "in any ranking of priorities for allocating R & D support... universities must come first", it declares that the federal government must make substantially greater investments in universities than it has in recent years. It also proposes a major new initiative to establish university interdisciplinary research centres.

Federal policies must, the panel says, recognize the cost of university research facilities. It proposes that the amortization period of buildings should be reduced from 50 to 20 years, while that for equipment should be reduced from 15 to 5 years. Recognizing widely felt alarm about the poor state of university buildings, it proposes a special facilities fund to be administered by the National Science Foundation, to disburse a total of \$10,000 million over 10 years. All proposals to the fund would be peer-reviewed and made on a 50-50 matching basis with non-federal funds. Tax incentives for industrial sponsorship of university research are also proposed.

The study panel also makes the now-infamous recommendation that reimbursements for administrative costs should be fixed at a uniform percentage, rather than being allowed to vary from one institution to another. This is the only recommendation that has been acted upon so far. It also proposes that grant durations should be increased to three or even five years, and makes other recommendations aimed at reducing administrative burdens on universities and, especially, researchers. New "portable" scholarships for the most talented 1 per cent of undergraduates and national merit-based multi-year graduate fellowships are also urged.

Tim Beardsley

US space

NRC board urges no skimping

Washington

THE Space Science Board of the National Research Council (NRC) has entered the debate over the future of the US space programme, calling for a "balanced fleet of launch vehicles", and urging a clarification of the goals of the manned and unmanned elements of the national effort.

In a statement released yesterday, the space science board concludes that by failing to identify accurately the resources needed by the manned and unmanned space programme, both have suffered. Thomas Donahue, chairman of the board and principal author of the report, says the country must decide how committed it is to a pioneering space programme. Donahue argues that if sufficient funds are not provided to carry out such a programme with "style and precision", then it should be abandoned. The space programme being a highly visible effort, failures brought on by inadequate funding or poor management are all the more glaring and devastating.

The space science board has been critical in the past of the decision to abandon the procurement of expendable launch vehicles before the shuttle became a proven, reliable system. The decisions not to pursue new unmanned launch systems and to commit the shuttle to carrying major scientific missions made space science unduly dependent on manned space-

flight. Had the shuttle lived up to its expectations, space science would not have suffered too badly, according to Donahue. But the Challenger accident underscores the fact that there has been no unmanned launch vehicle for major scientific missions for almost a decade, a "devastating" situation for space science.

There are seven scientific missions that were supposed to be launched in the next two years. Of these, only the Hubble Space Telescope and the Gamma-ray Observatory specifically require the lift capacity of the shuttle. The Galileo mission to Jupiter would have required the shuttle with its original trajectory, but may be able to achieve its objectives using a fuel-saving trajectory and a combination of the Titan 34D-7 rocket and Centaur G-prime upper stage, both soon to be available. While the shuttle provides a powerful launch vehicle and the possibility of in-orbit repairs, Donahue questions the wisdom of having a manned spacecraft carry a fully fuelled satellite booster rocket.

The space science board produced its statement following meetings last month and this. Board members felt that the Challenger accident made it imperative for NRC to make suggestions on the course of the space programme. According to Donahue, the board's statement went through the NRC report review process in "world record time". **Joseph Palca**

French science

Research directors form lobby

SOME 280 French directors of research — and the number is increasing daily — are making a public appeal to the government over what they consider to be swingeing cuts applied to science by the new Prime Minister, Jacques Chirac.

Last week, this new and spontaneous French pressure group published a half-page advertisement in *Le Monde*, the country's major daily newspaper, paid for



by themselves at over FF 400 (£40) a head, lambasting the government for applying over half the recent cuts in total government spending to one ministry, that for research. A further advertisement is planned in *Le Figaro*, an important right-wing paper read by many of Chirac's supporters, if further funds can be raised.

The petition's organizers, Paris astrophysicists Jean Audouze and Pierre Encrenaz, said last week that the response of the country's leading scientists to their hastily-organized appeal had been "very encouraging". Some 50–60 per cent of those polled had responded within a week with sums from FF 100 to FF 1,000 to support the campaign.

"It really means that the directors have been shocked by this government's actions", said Encrenaz. In terms of cuts in "autorizations de programme", a strong indicator for future budgets as it represents sums which may be planned to be spent next year and beyond rather than actual cuts in current spending, 54 per cent of the government's recent savings fell on research and development, even though that represents only 2.3 per cent of the country's gross national product. This was enough to stir researchers into action. But worse may be to come.

Chirac has now told ministers that he will quadruple the present cuts, when the 1987 budget is announced in September. It is not clear whether research will suffer further, but Chirac did say in a letter to his ministers last week that the "overriding

necessity" to make major savings in government spending meant that "it will be impossible to satisfy the many sectional interests, however legitimate their arguments, that will certainly protest".

It is difficult to see this statement as anything but a mandate to the already powerful ministry of finance and budget to ignore all pleas, including those made in the form of advertisements in *Le Monde*. However, French scientists continue to hope for a softening of the government's line, even though Chirac's frosty embrace will include, he says, a cut of 1.5 per cent in all civil service positions (except education). This will strike a blow at scientists just like those who, unaware of the new policy, signed the new petition: researchers from the "grandes organismes" research councils, the Centre National de la Recherche Scientifique (CNRS), the Institut National de la Santé et de la Recherche Médicale (INSERM), and the Institut National de la Recherche Agronomique (INRA) who recently became civil servants after a great political battle.

Some scientists, indeed, see this winning of civil servant status as the prime cause of their present trouble, for some of

the principal and hardest opponents of the deal were the senior officials of the ministry of finance. Only an intervention by the now virtually powerless President François Mitterrand secured researchers their cherished "security". Now the enlarged finance ministry is wreaking its revenge, the story goes.

Whether or not this particular story is merely paranoia among the scientists, there is certainly considerable evidence that research is now being singled out for harsh treatment in France, after a few years of exactly the reverse. Only university researchers outside the research council system seem to have been relatively protected in posts (as teachers) and budget; but this research sector, by common consent, represents the weakest part of French science. Even Chirac's most extreme scientific advisers before his election last March argued that the universities had to be reformed and strengthened before the research council system was attacked (their criticism was that the latter was too centralist); but even their caveats now seem to be being bypassed.

"There seems to have been very little thought behind these new policies — they are very hasty policies", says Audouze. "We hope our campaign will convince ministers that these decisions are jeopardizing French research." **Robert Walgate**

SDI

Abounding hypotheses floated

Washington

To students at the California Institute of Technology, satire has seemed the best way to express opposition to the Strategic Defense Initiative (SDI). Last month they held a competition for the most "destabilizing, unworkable or incredibly expensive" proposal to achieve SDI's goals.

The winner is planetary science graduate Greg Ojakangas. His proposal, estimated to cost \$457 million, involves developing a genetically engineered strain of pygmy hippopotamus. Hippos would be fed on a diet enriched with suitable radioactive materials and kept in cages with cathodes embedded in the walls. A continuous flow of electrons neutralizes the alpha particles emitted by the hippos' radioactive diet, forming helium and making the hippos lighter than air.

In the event of missile attack, an "optically thick cascade of buoyant hippos" rises to form a shield. Upon reaching the "hippopause", the height at which the mean hippo density (MHD) equals the atmospheric density, the hippos would fan out, forming a protective ROOPH (Readily Operative Overhead Protection by Hippos). In his proposal, Ojakangas reckons the hippos will remain buoyant for two days. After that, they will float gently to earth where they can be collected.

Second prize went to physics graduate student David Palmer, whose proposal calls for splitting the Earth into hemispheres at the Equator. A large bearing allows the two hemispheres to be rotated. When radar detects a flight of missiles heading over the pole, large motors rotate the two hemispheres 180 degrees in opposite directions.

After this manoeuvre, the eastern and western hemispheres have changed places, and the missiles will land on the country from which they were originally launched.

Other less serious scenarios suggested putting everyone in the United States aboard dirigibles (since "no dirigible has been shot down in 20 years"); erecting a cardboard shield as the final layer of a multilayer defence (cardboard is lighter than plexiglass); and using a series of lenses to focus the ability of the Bermuda Triangle to make things disappear.

The Caltech competition was inspired by a similar effort at Cornell University last fall. The winning entry at Cornell proposed using a particle beam of tachyons, the particles arising in certain superstring theories which travel backwards in time. This would provide a pre-launch destruction capability even after an attack had been initiated.

Joseph Palca

Supercomputers

India resents US strings

New Delhi

THE United States has imposed certain conditions on the sale of supercomputers to India. The offer was made by President Reagan ten months ago at the height of Indo-US cooperation, but the Pentagon subsequently raised objections.

Acting US Science Advisor Dr John P. McTague, who was recently in India, says the offer still stands but India must comply with a safeguards regime that has just been finalized by the US Departments of Commerce, State and Defense. According to McTague, the United States has not sold supercomputers outside the Western bloc, hence the safeguards. The conditions have been communicated to New Delhi and the ball is now in India's court.

The United States has simplified the procedures for the export of high-technology items since the two countries signed a memorandum of understanding in May 1985. Under the memorandum, the United States agreed to speedy clearance of high-technology equipment for civil and defence needs subject to assurances about its end use and a guarantee by the Indian government that such equipment would not be used for nuclear programmes or allowed to fall into the hands of the Soviets.

But the conditions for the sale of supercomputers go further. The United States is seeking assurance that a computer will be used for the specific purpose for which it is bought and not diverted for military or nuclear research. Programs to be run on the computer must have US approval, as must personnel with access to it. As well as the right of periodic inspection, the company supplying the supercomputer will have control over maintenance and repair and Indians will not be allowed to remove any part of the hardware or software.

Indian officials are studying the implications of the safeguard conditions and it is likely that they will be found unacceptable. "It would be demeaning to purchase a supercomputer under these conditions", one official said. "Inspection means that an American will be sitting at the computer centre checking every program we run. We should not accept this."

Indians have been interested in acquiring supercomputers for medium-range weather forecasting and monsoon research and possibly for computer-aided design of a light combat aircraft.

India has already entered into a \$26 million deal with Control Data Corporation (CDC) under which the state-owned Electronics Corporation of India will manufacture 600 units of Cyber-810 and Cyber-830 mainframe computers during the next nine years. India awarded the contract to CDC against stiff competition

from the French computer company Bull in the hope that CDC would also sell Cyber-205 supercomputers. Even if India accepts the safeguards, it is believed in India that the United States would be reluctant to supply the supercomputers because of India's opposition to the US

Japan Prize

Dutch winner attacks US policy

Tokyo

AT a memorial lecture on artificial organs by one of this year's winners of the Japan Prize, Japan's answer to the Nobel Prize, would one expect to see pictures of aircraft carriers and Trident submarines along with cartoons of star wars and the effects of nuclear winter? Dr Willem Johan Kolff, 1986 Japan Prize laureate for medical technology, apparently felt his lecture in Kyoto and Tokyo last month was a fitting occasion to lambast US defence policy and appeal for support for the anti-nuclear movement. But how does one turn a talk from kidney machines to killer satellites?

Kolff's speech began normally enough, with a historical review of the kidney machine he developed from 20 metres of artificial sausage skin in the Netherlands during the Second World War. With more than 66,000 Japanese now dependent on dialysis and the number doubling about every five years, there are many in Japan grateful for Kolff's invention and few would dispute his eligibility for a major international award.

From kidney machines, Kolff quickly switched to graver topics and a Rubens' picture was compared to the "life and death committees" that sprang up in the United States in the 1960s, when there were too few dialysis machines to go round and those to be saved had to be selected on the basis of age, occupation, number of children and so on. Then came a warning that the life and death committee is raising its ugly head again in the United States under the guise of "cost containment".

And why is cost containment necessary? In sailed slides of aircraft carriers and Trident submarines, along with the message that the United States is spending too much on defence and too little on treating its fellow citizens. In contrast, Kolff portrayed Japan as a haven where low defence spending allows high industrial productivity and where all who need kidney machines get them. But with kidney transplants virtually unobtainable, Japan is hardly a paradise for patients with renal disease (see *Nature* 313, 338; 1985). And what of the defence the United States

bombing of Libya.

If the United States does refuse to sell supercomputers, India will turn to Japan or the Soviet Union. The Department of Electronics has already purchased four S-1000 supercomputers from NEC of Japan for its nationwide information network. And the Soviet Union has agreed to sell, without strings, its Elbrus parallel processing machine as soon as it leaves the factory early in 1987. **K.S. Jayaraman**

has provided for Japan for 40 years?

Kolff then turned to the membrane oxygenators developed from the artificial kidney after it was noticed that blue blood passing through the dialyser turned red. Who needs oxygenators? The 50,000 victims of nuclear war with scorched lungs. But, asserted Kolff, nobody would be interested in artificial lungs because they would all die anyway from the effects of nuclear winter. The stage now set, Kolff leapt back and forth from artificial hearts to nuclear fire power and star wars to artificial eyes and ears. In his abstract he threw in the greenhouse effect for good measure, but his listeners were spared the assertion that "the polar ice caps will melt" and "the coastal plains of Japan will be flooded". Kolff closed by urging the audience to join anti-nuclear movements such as International Physicians for the Prevention of Nuclear War, the controversial winners of last year's Nobel Peace Prize, and ended by asking "what good will an artificial heart do us if we are all vaporized?"

The Japan Prize is awarded by the Science and Technology Foundation of Japan, a non-profit organization under the direction of the Japanese Prime Minister's office, and is intended to recognize significant and original achievements in science and technology that contribute to peace and prosperity for mankind. The foundation's dedication to peace provided Kolff with the platform for his anti-nuclear appeal, and as Japan is edging towards a decision to participate in the Strategic Defense Initiative under the enthusiastic backing of Prime Minister Yasuhiro Nakasone, Kolff's speech was timely. But will this lecture help elevate the Japan Prize to a comparable level of recognition with the Nobel Prize, one of the unstated aims of its founders?

David Swinbanks

Correction

IN the article "Making radiation understood" (*Nature* 321, 195; 1986), the relative value of curies and becquerels is incorrectly given: it should read $1 \text{ Ci} = 3.7 \times 10^{10} \text{ Bq}$. □

Japan's forests

Welshman begs respect for trees

Tokyo

C.W. NICOL must be Japan's most famous Welshman — indeed perhaps the only Welshman the average Japanese has ever heard of. A string of popular books on the natural world published in Japanese plus, at 45, an autobiography which includes words like "Pwllheli" which no Japanese (or Englishman) will ever be able to pronounce have made his name. Most recently, he has been in the public eye with an impassioned plea to the Forestry Agency to spare the forests that surround his home deep in the mountains of central Japan. The white paper (policy document) recently released from the Forestry Agency, which is assiduously cutting down the forests around Nicol's home, reveals that even without an infuriated Welshman to deal with, the Forest Agency, Japan's forests and the forestry industry are in dire straits.

Trees take a long time to grow and it is not easy to keep forestry policy in tune with a rapidly changing world. Some sympathy must thus go to the Forestry Agency. But there are many who have been telling the agency that its policies are mistaken for at least the past fifteen years.

The Forestry Agency found itself at the end of the Second World War with many mountains denuded of timber. Plantations, largely of *sugi*, the Japanese cedar, were begun. Then Japan entered its high-growth era and it seemed that the demand for timber would never stop growing. Clearance of natural forests began on a massive scale and huge numbers of cedar were planted.

Long before any of this timber was ready for the market, very much cheaper timber began to pour in from abroad. At the same time, new materials began to be used to build houses. Then Japan's growth began to slow; the Forestry Agency and

private landowners found themselves with huge tracts of forest on their hands that may never be sold at a profit. Much the greater part of that forest is still young enough to need regular thinning and care to remain healthy. But instead, with no profit in sight, huge areas have been left to rot. The white paper admits that 40 per



Natural forest left along water courses in the national park near Tokyo.

cent of man-made forests that need thinning are not being attended to.

Part of the reason why home-grown timber is expensive is that it is difficult to take out trees from Japan's steep mountain sides. Roads capable of carrying heavy timber lorries must be built and aerial ropeways set up. That adequate, cheap technology is not available is something critics have been saying for years. Nor, critics say, was adequate thought given to the suitability of cedar in the rush to plant forests.

Plantations in poor locations have been hit by mice, frost and insects, while too much reliance may have been placed on cedar. There is still plenty of demand for broad-leaved trees. The white paper argues for diversification, which critics have been demanding for years. Meanwhile, profits are being made by cutting existing natural broad-leaved forests, including trees hundreds of years old. That is what angers Nicol.

Letters of protest to the government from individuals do not normally evoke much response, but whether out of respect for a foreign "guest" or from sensitivity to foreign criticism, or because Nicol's letter was also published in the *Yomiuri* newspaper, the Forestry Agency director-general personally promised (according to *Yomiuri*) that the beeches and birches on the mountains around Nicol's home would not be cut. But this, it seems, was too good

to be true. Forestry officials who visited Nicol's home revealed plans for cutting the forest on a scale beyond his worst imaginings. The only thing they appeared to take note of, he says, was not to litter the forest with discarded chain-saw blades.

But Nicol has discovered he is not alone in his love of the forest. Hundreds of letters of support and telephone calls have come to his home. The burgeoning enthusiasm for forests is also clearly recognized in the white paper, as it was in an advisory report to the agency published last August. Many Japanese have of course been proclaiming for years that they have a unique sensitivity to nature, but what they mean is cherry blossoms in parks, pot plants outside their homes and cut flowers impaled on spikes in vases and twisted into miniature imitations of nature. Love of nature in the raw, complete with the mosquitoes, snakes and bears that inhabit Japanese forests, is a very new phenomenon. Many new organizations concerned to protect forests and town greenery have sprung up recently, reflecting a change in Japan's society away from pure economic growth to concern for the quality of life. Gradually they are beginning to join forces but none is yet big enough to exert a powerful influence. The Forestry Agency has responded by decreasing the area of forest that can be cut at one time and by increasing emphasis on the selective cutting of forests so that natural regeneration can follow.

Catering for this new interest in forests would not be so difficult were it not that the Forestry Agency is expected both to be financially self-supporting and also to ensure the stability of mountain villages whose only source of income is from forestry. It is not surprising then that the white paper proposes that "the people bear some of the financial burden to improve the nation's woodland". A tax on water resources has been suggested as a way of financing protection forests planted to prevent flash floods. The tax is opposed by industry.

For all the letters he has received, Nicol is not going to try and join in public protest movements. He intends instead to charter a plane and photograph the forests near his home from the air and then from the ground and use his pen and the pictures to publicize the forest's plight. For changes in forest policy there is still time, for Japan is lucky in that large areas of natural forest do still remain — any European country with such forests would consider itself blessed indeed. Whether public awareness of the immense value of the forests will grow fast enough to prevent them disappearing remains the real question. And on top of that there may be some other problems — the white paper is the first to admit that acid rain may be causing forest damage in Japan.

Alun Anderson

Widespread forest

JAPAN'S total land area is 378,000 km². Of this, 250,000 km² is forest: by no coincidence this is a figure very close to the area that is mountainous, 268,000 km², and cannot be used for agriculture or habitation. Almost a third of the forest is owned by the state and a little over a third is man-made, nearly half of it Japanese cedar and a quarter *hinoki*, Japanese cypress. Various kinds of national park and nature park account for a remarkable 54,000 km² of land, an extremely high percentage. But only a small fraction of the park area is completely protected from interference; in other regions, permission can be given for selective cutting, or clear cutting, of forests. □

Safety of French nuclear plant

SIR—The safety record of COGEMA's reprocessing plant UP-2 at La Hague may be better than that of British Nuclear Fuel Limited's Sellafield plant in Britain (*Nature* 320, 204; 1986), but is it really that much better?

In 1984, collective radiation exposure was the highest in the history of the La Hague plant (728.1 men-rem), surpassing the figure of 1981, when a fire in a waste silo caused significant contamination (and one of the exposed workers caught a dose of 5.7 rem).

The radiological situation on and around the site is anything but stable. The average tritium content in the water of a stream in the area increased 13-fold between May and December 1984, and average β -activity in another stream by a factor of 60 in the last four months of 1984. The official explanations for the presence of unusual quantities of tritium (seasonal rainfalls and air humidity) and β -activity (mainly cobalt-60 washed off the spent fuel cask storage site) are meagre.

There have been several other leaks and incidents in the past few years. The most recent was what COGEMA called a mini-leak in November 1985, when 400 m³ of "slightly radioactive liquid" was spilled into the environment.

COGEMA's flexible approach to the "nominal capacity" of the plant is significant. In 1976, the official *Revue Générale Nucléaire* says of the processing of fuel from light-water reactors (LWR) that France had completed the UP-2 plant at La Hague by an oxide headend called HAO (Haute Activité Oxydes) which would reach its nominal capacity of 800 tonnes of oxide a year around 1979–80. In 1979, the actual throughput of the plant was 79.4 tonnes of oxide fuel and, in 1980, 104.9 tonnes were reprocessed, 10 per cent and 13 per cent respectively of the "nominal capacity".

In 1978, the optimistic goal of 800 tonnes a year was abandoned and the "new" nominal capacity was fixed at only 400 tonnes a year. In 1982, the Castaing commission, which was asked by the minister of industry to analyse the situation at La Hague, stated that it was more likely that HAO's capacity would be limited to 250 tonnes a year, and COGEMA said in its 1983 annual report that "UP-2 will be capable of reprocessing 250 tonnes a year of LWR fuel, once the reprocessing of other fuel types has been discontinued".

Since 1984 COGEMA has returned to the figure of 400 tonnes a year, as if there had never been anything else. COGEMA proudly announced that 418 tonnes had been reprocessed between November 1984 and November 1985, a performance "beyond its normal capacity". In other words, it took COGEMA ten years to

reach 52 per cent of its initial design capacity.

Most of the LWR fuel reprocessed at La Hague was not French but foreign. By June 1982 only 11.5 tonnes (2.6 per cent of the total) were irradiated in a French reactor (Fessenheim). Two years later (June 1984) 82.3 tonnes of EDF (Electricité de France) fuel had been reprocessed and, by the end of 1985, the total quantity had reached 290 tonnes, 22 per cent of the 1,338 tonnes of oxide fuel reprocessed at HAO by the end of 1985, according to Maurice Delange, head of COGEMA's reprocessing branch.

The foreign fuel came from West Germany, Japan, Belgium, Switzerland, the Netherlands and Sweden. COGEMA argues that there was not enough French fuel available, although EDF has a contract with COGEMA for the reprocessing of 5,344 tonnes of LWR fuel; its continuing hesitation to commit itself fully to the reprocessing strategy of the Commission à l'Énergie Atomique (CEA) made COGEMA look for additional contracts with foreign clients. According to M. Delange, COGEMA's offer of an additional 1,000 tonnes of capacity for 1986–90 on the international market led to some possible new contracts with the same clients.

The time has passed when CFDT union workers were "not slow to report accidents to their union", as Walgate puts it. Until 1981, CFDT used to be by far the strongest union in La Hague. But after the socialists came to power, it lost many members and half their votes at the elections of union representatives, to the profit of the conservative pro-nuclear FO (*Force Ouvrière*). The union's critical point of view has been turned against it. To be against foreign reprocessing contracts and in favour of the development of alternative waste management strategies has been regarded as "destroying the working tool", as a CFDT worker explained. The "chantage à l'emploi", literally employment blackmailing (threat of redundancy), has been used extensively by the reprocessing lobby, including competing unions. It works well in an area of high unemployment (8,000 people are registered unemployed in Cherbourg), where the only two important employers are the French Navy and COGEMA.

In France, independent information on the nuclear industry and especially on reprocessing is less easily available than ever. The nuclear lobby can give a more "open" appearance and its efforts to "inform" the public, over the past ten years, have indeed been enormous. Workers keep their mouths shut; nobody wants to lose his job.

One of their problems is the lack of

strong national support; the national CFDT nuclear branch was "decapitated" in 1981, when highly qualified leaders took over the management of the Agence Française pour la Maîtrise d'Énergie, a state energy management agency. For the CFDT section at La Hague this was a second blow. They had had to find new leaders when the organizers of the 1976 strike (to protest against existing safety conditions) left La Hague when COGEMA (100 per cent owned by CEA) took over the management of the plant; they feared repression from a new management which, unlike CEA, was not exempt from private law.

Today, union workers do not want to be telephoned for fear of telephones being tapped, letters stay unanswered and they prefer not to be mentioned in public at all. The few who remained active are gradually giving up; "If they want to eat plutonium, I can't help it", as one stated sarcastically. Another could not find anyone to help write, print and distribute an internal flyer. And nobody wants me to talk about all this...

MYCLE SCHNEIDER
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Pre-embryos

SIR—Anne McLaren (*Nature* 320, 570; 1986) states that the human product of conception before the 14th day of intrauterine life should not be called an "embryo" because the cells are destined to develop into both the fetus and the placenta. By implication, experimenting with or throwing away a conceptus less than 14 days old does not constitute ill-treatment of a human embryo. It is maintained that the use of such terms as "pre-embryo" for these earliest stages of development is not euphemistic or "cosmetic", but scientifically valid terminology.

Later in intrauterine life, when obstetricians and perinatologists strive to save a life at risk, the togetherness of the baby and its placenta is emphasized by the currently fashionable term "feto-placenta unit". The placenta is then an essential organ that is discarded only when the baby can manage without it. It happens that before and immediately after implantation the cells that will form the placenta outnumber those that will form the baby, and one cannot look at an individual cell and recognize the organs that will be derived from its progeny.

Words like "pre-embryo" may have scientific precision, but they should not be used to foster the delusion that those few cells are anything less than a young feto-placental unit.

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Disappointment over double quasar

There now seems a high chance that what seemed, two weeks ago, to be a particularly interesting double-quasar image may simply be a record of two independent objects.

Two articles elsewhere in this issue (pp. 419 and 420) provide what appear to be plausible explanations of the remarkable report by E.L. Turner *et al.* (*Nature* **321**, 142; 1986) of the apparent identity of two quasar images separated from each other on the sky by more than two minutes of arc (2.6 minutes, to be precise). The significance of this report was quickly appreciated not merely by its authors but by many others. Although there has accumulated since the first observation in 1979 a handful of cases in which the image of a single quasar is split into two or even three by the interposition of a massive galaxy along the line of sight to the distant object, the angular separations previously reported have been very much smaller than that of the pair of images measured by Turner *et al.*, a few seconds of arc rather than a few minutes.

This week's authors, both Princeton colleagues of Turner, explore the possibility that the very large separation may be caused by the interposition either of a very massive black hole or, alternatively, of one of the galactic structures called a cosmic string. Readers will note that their calculations, interesting in their own right, are at once tentative and suffused with a sense of what good luck it must be that observations should have led so simply to such striking inferences. Now, unfortunately, it appears that these endeavours may have been misplaced.

In a field where it is never easy to be sure that quasar images have been accurately identified with each other, it is too soon for certainty. But stimulated by the report of Turner *et al.*, others have looked at the components of the supposed double quasar in spectral regions other than that on which the original identification was based, and they find enough discrepancies to suggest that the apparent identity of the two objects is in doubt.

Before jumping to the conclusion that Turner *et al.* are in some way to blame for having published their data too quickly, or that they have made an elementary mistake that might have been avoided by taking greater care, readers should consider carefully what is involved in the identification of a gravitationally split quasar image. That an intervening massive object can split one image into two or more follows from the way in which, in relativity, the gravitational field of a massive object distorts the usual straight-line paths open to light. The magnitude of the effect

and the apparent separation of the two images depend on the mass of the intervening object. The known cases of split quasar images require intervening masses comparable with those of galaxies. Turner's separated images would require more massive objects, whence the speculation about black holes and cosmic strings.

To establish that a close pair of quasar images has indeed been generated by the interposition of a gravitational lens is, in reality, quite difficult. The first test is that the two images should have the same measured red-shift (which is not affected by the bending of the gravitational lens). One difficulty is that the red-shifts are usually so large that it is hard to tell which atomic transitions are ultimately responsible for the spectral lines observed in the emission from the quasar. Another is that the lines, even if accurately identified, may be so broad that the accuracy of inferences about their displacement is bound to be limited. Turner's two images were reported to have a red-shift defined by $z = 1.012$ on the strength of an emission line from magnesium which, while normally in the ultraviolet, has been displaced by the recession of its source to the middle of the visible light spectrum.

Those who search for double-quasar images also pay close attention to the general characteristics of the spectra of the two candidate images, but there are some important complications. Thus the spectra of quasars can vary rapidly on quite short timescales. Most of the variations show up in the continuum flux, but there may also be substantial variations in the strength of emission lines in the spectra. Because one of the consequences of the effect of a gravitational lens is that different images of the same quasar may reach the observer by paths which differ considerably in the traverse-time of light, there is room for some latitude in the comparison of the spectra of two images. In Turner's case, the two light paths may have differed by as much as 1,000 light years, ample to account for the spectral differences he and his associates reported in the two quasar images.

The new development is a set of measurements by P.A. Shaver and S. Christiani of the European Southern Observatory in a part of the spectrum extending towards the infrared from the longer wavelength limit of the range covered by Turner *et al.* An article describing these measurements will appear in *Nature* two weeks from

now. In this previously unexplored region of the spectrum of the Turner quasar, there appear to be pronounced differences in the intensity of certain hydrogen lines. Readers will have to judge for themselves, when this evidence appears, whether the quasar images are indeed produced by a gravitational lens from the same single distant object. Advance notice of these measurements is being provided now, with the consent of its authors, merely because of the excitement generated by the original measurements.

If Turner *et al.* were unlucky in their choice of a spectral range in which to carry out measurements of the components of the double quasar, there will be a widespread sense of disappointment that a potentially fascinating phenomenon has so quickly vanished. But that does not imply that the two quasar images concerned are without interest. The supposition that they might be gravitationally split images of the same object seems first to have been raised by Paczynski (*Nature* **319**, 567; 1986) on the basis of measurements originally due to C.H. Hazard.

If the two images are now gravitational artefacts, then they are most probably distinct quasars (with nearly identical red-shift) which are also members of a distinct galactic cluster. But the measured difference between the velocities of the two images is less than 200 km s^{-1} , which is less than the spread of velocities found in typical galactic clusters, and which may therefore suggest some special relationship between the two quasars. That would be an interesting if less spectacular development in its own right, one that would no doubt bring comfort to those who, like H. Arp, have long argued for some systematic relationship between the positions of quasars in the sky.

Meanwhile, the urgent need is further to test the relationship between the two images of Turner's quasar. Detailed study of the relative intensity of the iron and magnesium lines in the two images will no doubt persuade most people one way or the other. When all this has been done, there will remain one logical ambiguity. If the travel times of widely split quasars may be of the order of 1,000 years, and very much greater than the timescale of substantial variability of individual quasars, will it ever be possible to prove (or disprove) that such widely split images are caused by gravitational lenses, however massive or peculiar? □

AIDS virus

More and better *trans*-activation

from A. Burny

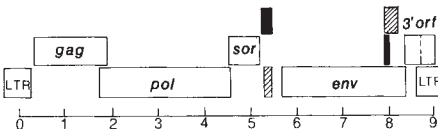
THE virus that causes acquired immune deficiency syndrome (AIDS) is a sophisticated weapon aimed at the human T4 lymphocyte, a key cell of the immune response. This highly cytopathic virus, called HTLV-III, LAV or, most recently, HIV (see ref. 1), is endowed with the means to stimulate its own replication in an infected cell. One gene involved in this autostimulation is *tat III* (refs 2-4), a bipartite gene encoding a protein that interacts with receptor sequences near the upstream (5') end of the viral messenger (m)RNA. On page 412 of this issue⁵, Bill Haseltine and his colleagues describe the discovery of a new bipartite gene, *art*, of HIV that is also involved in autostimulation. The product of the gene activates the expression of the *gag* and *env* genes of HIV (encoding, respectively, the capsid and envelope proteins) and thereby regulates virus replication.

The discovery of *art* (for anti-repressor translation) is an intellectual and technical achievement. Haseltine's group recently investigated⁴ the mechanism of *trans*-activation by the *tat III* gene product and observed that elevation of long terminal repeat-directed gene expression in cells expressing the *tat III* protein reflects an increase in protein synthesis without a comparable rise in the steady-state level of the respective mRNAs. Deletion analysis of the HIV long terminal repeat demonstrated that the sequence necessary for *trans*-activation (the TAR sequence) is located between nucleotides -17 and +80, that is, 3' (downstream) from the mRNA initiation site (the cap site). Taken together, the lack of effect of *tat III* on viral mRNA concentration and the presence at the 5' side of viral mRNAs of nucleotide sequences responsive to *tat III* protein regulation prompted Haseltine and co-workers to establish experimentally that *trans*-activation by *tat III* is mediated via a post-translational mechanism⁶.

Continuing their deletion analysis of the HIV proviral genome, Haseltine and colleagues have now identified a seventh viral gene. Their approach consists of the transfection of matched cell lines with intact or deleted proviral DNA constructs and determination of the amounts of virus and viral antigens produced. It became obvious that a new gene was involved when deletions located in the 3' region of the first exon of the *tat III* gene, but not extending into the *env* gene, prevented virus production even in the presence of a functional *tat III* product supplied in *trans*. A second set of mutations (insertions and deletions) located further down

the proviral DNA to the 3' side of the second exon of *tat III* and overlapping *env* yielded proviruses that failed to express viral *gag* and *env* proteins. Finally, new plasmids designed to express the putative new open reading frame but not *env* were demonstrated to allow *env* production by proviruses containing mutations in the new open reading frame. As the phenotypes induced by both sets of mutations performed are similar, Haseltine and colleagues conclude that they have identified a new gene.

The protein product of the gene, 116 amino acids long, has no effect on viral



Map of the genes encoding the AIDS virus. Black boxes, the two coding exons of the *tat* gene; hatched boxes, those of the *art* gene; *pol*, *sor*, genes for other viral proteins; *orf*, open reading frame. Scale, kilobases.

mRNA concentrations but is required for expression of the HIV *gag* and *env* proteins. The inescapable conclusion of these experiments is that the *art* gene product specifically allows translation of HIV *gag* and *env* mRNAs, thus unlocking the block imposed on virus expression. The discovery of *trans*-activation of retroviral long terminal repeats or mRNAs will be important for understanding virus persistence and latency, cell killing and cell transformation.

The *tat* and *art* gene products are clearly potent regulators of viral gene expression, and are most probably involved in the successive cycles of latency and expression

that characterize the life-span of the AIDS virus. Fine tuning of *tat* and *art* genes must allow silent accumulation of *gag* and *env* mRNAs. Expression of *tat* and *art* proteins switches on a burst of expression of *gag* and *env* components that initiates virus production. Such a mechanistic design predicts that *tat* and *art* mRNAs must be translatable in conditions that preclude translation of *gag* and *env* mRNAs. Detailed analysis of the 5' end of the corresponding mRNAs, and identification of the *tat* and *art* protein interactions with their target nucleic acids, are urgent tasks.

As Haseltine and co-workers point out, the new data are highly suggestive of the existence of an early-late switch in HIV infections and in infections mediated by similar agents such as visna virus and caprine arthritis encephalitis virus. The two latter systems are characterized by persistence and latency⁷⁻⁹, and *trans*-activation has been demonstrated in visna virus-infected cells⁹.

Retroviruses, DNA viruses and other systems involving production of a lethal product obey the biological paradigm that production of the product is best achieved if delayed to late stages of the life-span of the cell. Regulation of translation of accumulated mRNAs is an elegant manner of complying with this requirement. □

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Gravitational waves

Observatories of the future

from M.G. Edmunds

THE direct observation of gravitational waves, the ripples in space-time generated by the motions of masses, is one of the last-known windows that remains to be opened on the Universe. Although detections have been promised within five years for at least the past fifteen years, it does at last seem that the hopes will develop into real data within six years from now, as concluded at a recent meeting* by members of groups working on the problem.

*The Interferometric Gravitational Wave Detectors meeting was held at Cardiff University on 17 February 1986.

This optimism springs from a consensus on the theoretical estimates of the expected strength of cosmic sources of gravitational radiation, as well as growing confidence that the technological advances required for interferometric detectors of sufficient sensitivity can be made.

An interferometric gravitational-wave detector (see Fig. 1) basically consists of three suspended masses arranged at the corners of an L. The two arms of a Michelson interferometer formed with mirrors attached to the masses measure the rel-

ative lengths of the two sides of the L. When a gravitational wave passes the detector it moves the masses on one arm apart and the masses on the other arm closer together, resulting in a shift of the interferometer fringe pattern. A parameter h is used to characterize the magnitude of the wave's interaction, defined as the induced difference in length of the two arms divided by the length of a single arm. Just to begin to see sources, values of h as small as 10^{-20} will probably need to be

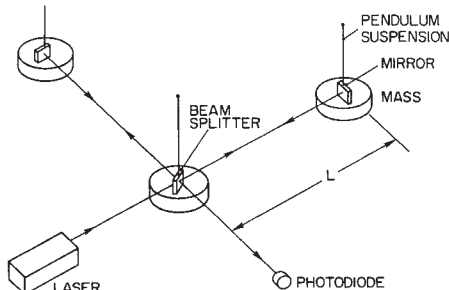


Fig. 1 Basic principle of the laser interferometer.

detected. Really useful astrophysical information will come when h can be measured at the 10^{-22} level. Because even the former of these limits implies measuring length changes of 10^{-2} fermi (about 1/100 the radius of a nucleus) in an interferometer with an arm length of 1 km, it is clear that the technological problems are considerable. Interferometric detectors work at wave frequencies within about a decade of 1,000 Hz, and are limited to optical or near-infrared light by consideration of the optic surface, the noise source and the photon detector.

As summarized at the meeting by Kip Thorne (California Institute of Technology; Caltech) the sources most likely to be seen as single events involve collapse of the core of a single star in a supernova explosion or coalescence of a close binary system of compact stars. Generation of gravitational waves is a strong function of the deviations from spherical and axial symmetry occurring during the collapse to form a neutron star or black hole, and the uncertain magnitude of these deviations leads to uncertainty in the predicted wave fluxes. A sensitivity of h of the order of 10^{-22} may allow collapsing black holes of 100 solar masses to be seen throughout the entire Universe accessible within the light-travel time since the Big Bang. Non-axisymmetrical neutron-star collapses may be seen out as far as galaxies in the Virgo cluster, yielding an expected several events per year. An advantage of interferometric detectors over resonating solid-bar detectors is the spectral information that can be deciphered. Recent numerical collapse calculations suggest very characteristic waveforms for both black-hole and neutron-star collapse; indeed detection of the wave 'signature' of a black-hole collapse may be our only way of unambiguously demonstrating the existence of

black holes. If the supernova generating the waves is also detected visibly, the relative arrival times of the light and gravitational waves are a strong test of gravity theories. General relativity predicts the same speed of propagation, while some rivals predict differences of the order of one part in 10^6 ; the observations should place limits of the order of one part in 10^{10} .

Two neutron stars spiralling into each other should certainly be visible in gravitational waves at the Virgo distance if h limits are pushed to 10^{-22} . The stochastic background of gravitational waves left over from the early Universe may also be detected, perhaps allowing some probing back to the Planck era ($t \sim 10^{-43}$ s) where some estimates place the decoupling of gravitational radiation, or to later eras when the perturbations in density that eventually gave rise to galaxies were generated. Present-day regular periodic sources (such as fast-spinning pulsars) may be observed to much smaller h than 10^{-22} by integrating over many periods.

R.W.P. Drever (Caltech and Glasgow University) outlined some of the neat experimental techniques that have made the interferometric detectors a practical proposition since the first experimental prototype built at the Hughes Aircraft Corporation in 1971. The sensitivity of the detector increases with the arm length. The effective length of the arms is increased either by using multiple reflections to give multiple parallel paths along the arms or by making the arms into resonant cavities, effectively bouncing the light backwards and forwards many times within the period of a gravity wave. The programme in Germany (like that at Massachusetts Institute of Technology, and described by W. Winkler, Max-Planck Institute, Garching) uses a multiple path design of total path length 100 km, possibly in a three-arm triangle configuration. The current experimental rig in Glasgow has an arm length of 10 m, and the rig at Caltech a length of 40 m, both rigs using the resonant cavity approach in preparation for much larger instruments. The development of a servo-loop system by Drever's group to stabilize the laser frequency in these interferometers has led to a standard technique for laser stabilization in many other applications — surely an object lesson for those who doubt the technological advantages of pursuing 'pure' science.

A new technique to use a phase-modulation system to recycle the light used in the interferometer in such a way as to re-

inforce the laser light feed may reduce the required laser power by a factor of 100, which will require single-mode powers of perhaps 20 or 30 W — a not unreasonable target. Particularly useful in reducing the requirements of laser power is the reported development of robust mirrors with reflectivities of the order of 0.99995. In the more distant future there are real hopes of reducing the effects of photon noise by using a technique termed squeezed states, recently demonstrated experimentally at Bell Laboratories, which attempts to circumvent some of the limitations imposed by the uncertainty principle by careful choice of exactly what quantities are measured.

The practical realization of a working gravitational-wave observatory (see Fig. 2) is most likely to come first from the US and the Glasgow groups. The US project for a laser interferometer gravity-wave observatory should result in two 4-km instruments, one in Maine and one in California. The UK proposal is for a 1-km instrument in Scotland, to be fully operational in its first stage within about six years. These would be true 'observatories',

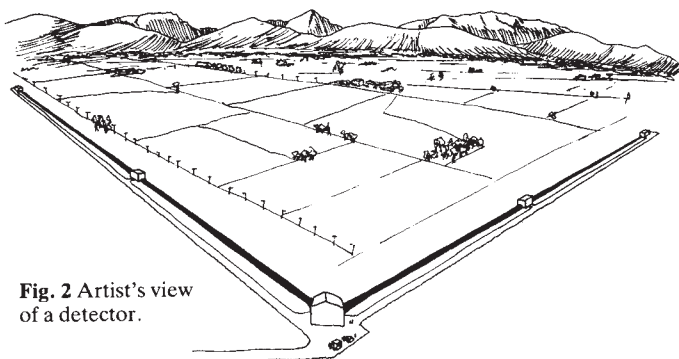


Fig. 2 Artist's view of a detector.

as most of the cost is in the provision of kilometre-length high-vacuum pipes for the interferometer arms. The pipes would be about 1 m in diameter and would work at vacua of better than 10^{-8} Torr. These pipes will be able to carry several interferometers simultaneously, providing useful coincidence tests against seismic or other instrumental disturbances.

The value of international collaboration in gravitational-wave research was unanimously agreed. Apart from economies resulting from shared technology, there is a real need for several detectors both to obtain directional information and to eliminate spurious signals by correlation of data from different sites. The gravitational waves sweep past and through the Earth, and (as emphasized by B.F. Schutz, Cardiff University) the four principal detectors proposed can be optimally oriented to give good sky coverage and will maximize the rate of detection of the (initially) few events per year. □

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Developmental biology

Embryology goes fishing

from David Marcey and Christiane Nüsslein-Volhard

In all multicellular organisms, embryonic cells with different developmental potentials arise from a series of cleavage divisions that begin with the first division of the nucleus of the fertilized egg. In some animals, for example the nematode worm *Caenorhabditis elegans*, the developmental fate of a particular embryonic cell is strictly tied to its ancestry, whereas in other systems (for example, the fruitfly *Drosophila melanogaster*), positions and/or cell interactions are instrumental in determining initial restrictions in developmental potential, although these restrictions can be inherited by daughter cells through subsequent cell divisions. But the relationship between embryonic cell fate and cell lineage has been examined extensively in few vertebrates, so the recent report by Kimmel and Warga¹, describing some early cell lineages in the zebra fish *Brachydanio rerio* (a long-time aquarium favourite), is therefore welcome.

Although the various organisms examined to date differ in the degree to which embryonic cell determination is linked to cell lineage, there is no reason to expect that, at the cellular level, fundamentally different determination mechanisms are used during the embryogenesis of diverse taxa. Significant increases in our understanding of development in different species are required before such comparative problems can be addressed. Recently, progress in elucidating embryonic cell-determination mechanisms has been more rapid for invertebrate systems (especially flies and nematodes) than for vertebrate systems. This is partly because of the higher complexity of, say, mouse than worm development, but has also resulted from the lack of a suitable vertebrate system in which the power of a genetic approach to ontogeny can be brought to bear.

In *Drosophila* and *Caenorhabditis*, the discovery of developmentally important genes by the recovery of mutations, followed by their molecular analysis, is permitting significant glimpses into the mechanisms responsible for pattern generation, including those involving cell-lineage restrictions. Are these mechanisms also operative in vertebrates? Two approaches are likely to help answer this question. One involves the analysis of vertebrate DNA sequences that show significant sequence homology with genes known to figure importantly in fly and/or worm development. The other is to perturb development genetically in a vertebrate, to screen systematically for embryonic defects and then to compare the classes

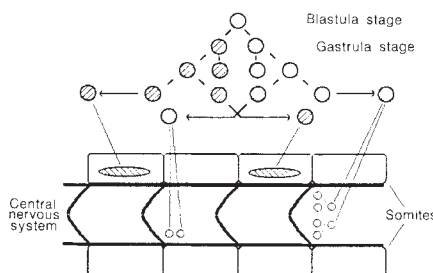
of mutant phenotypes obtained with those available in other organisms. Such comparative, genetic embryology should provide valuable information about the evolution of developmental systems.

Fortunately, some of the genetic techniques devised by George Streisinger² (Fig. 1), coupled with the features of the zebra fish that provide for superb embryological analysis, make this species an ideal vertebrate for the genetic analysis of

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Fig. 1 Cloned zebra fish embryos.

Fig. 2 Schematic, simplified representation of sample cell lineages in the zebra fish embryo (the numbers of cell divisions shown are not necessarily real). Striped cells are of a muscle lineage, dotted cells are of a neural lineage. A blastula cell may give rise to both muscle and neural cells, but at the gastrula stage, separation of these lineages occurs. Subsequent divisions produce cells which can separate (arrows) and populate different levels along the antero-posterior axis. In the case of some neural cells, further divisions yield cells that lie on both sides of the nervous system midline.



development. Mutagenesis screens, together with embryological analyses like those of Kimmel and Warga, promise to advance our knowledge of vertebrate ontogeny.

Taking advantage of the transparency, small size and speedy development of zebra fish embryos, Kimmel and Warga¹ injected a conjugate of the fluorescent label rhodamine and the histochemical marker horseradish peroxidase (or fluore-

scent label alone) into single cells of the blastula and followed the subsequent fate of their descendants in both living and sectioned material. Confirming previous marker-injection³ and genetic mosaic⁴ studies, progeny of single blastomeres were seen to disperse throughout the embryo and to participate in the development of diverse tissues such as muscle, neural and epidermal tissue. In contrast, a detailed analysis of individual gastrula-stage lineages within a marked clone revealed that, with few exceptions, progeny of gastrula cells are confined to a particular tissue.

These observations fit well into the classical embryological framework for fish development. In teleosts such as *Brachydanio*, pronounced cellular mixing takes place during the spreading movements of the embryonic disk over the yolk that precede and accompany gastrulation, termed epiboly. A result of this epibolic spreading is the juxtaposition in the blastula of cells having different origins. During and after this mixing, formation of both germ layers and the rudiments of primary organs occurs. Thus, although not surprising, Kimmel and Warga's new result helps to define the stage at which initial segregation of tissue-specific lineages occurs. The observed clonal restrictions might not simply be caused by spatial constraints because the authors have found that separate tissue-specific lineages sometimes produce cells that are near neighbours; gastrula-stage lineages are segregated into tissue types even when there is substantial cell mixing and division throughout the period of observation. These segregations could then reflect heritable choices in cell fate made by individual gastrula cells (see below).

Several clonal patterns in the central nervous system deserve special comment. Kimmel and Warga observe that cells arising from a common neuronal precursor can populate disparate axial levels. Data from one embryo show extensive antero-posterior movement of the descendants from sibling mid-gastrula neural progenitors such that clonal relatives are present as four clusters separated by as many as seven segment widths in the 24-hour-old embryo. One possible interpretation of this result is that the segmental identity of individual neural cells is assumed after a commitment to make nervous tissue. A test of this interpretation would involve making appropriate lesions to prevent the normal migration of neuronal precursors and then following the fate of thwarted cells. Kimmel and Warga found marked, if somewhat variable, periodicity in the clustering of clonal relatives in the spinal cord; sometimes relatives in adjacent segments differentiate into morphologically similar neurones. Another striking observation was the dispersal of some sibling

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neural progenitor cells across the embryonic midline after migration of parental cells to a particular axial level. Judged by their morphology and rostro-caudal position within a particular segment in the spinal cord, these neurones appear homologous. The intriguing observations that clonal relatives in different segments and in separate halves of the embryo can differentiate similarly demands thought on the relationship of this differentiation to lineage.

Based on their analyses, Kimmel and Warga suggest a sequence of diverging lineages associated with cell-fate determinations in zebra fish (Fig. 2): although blastomeres mix extensively and are pluripotent with respect to the fate of their descendants, the first divisions of the early gastrula produce tissue-specific lineages; the next divisions produce cells that contribute to different axial levels; and subsequent divisions, at least in the central nervous system, yield sibling cells that form the bilateral halves of the brain and spinal cord. The tracing of cellular pedigrees with injectable markers⁵ clearly yields much information about the embryonic fates of clonally related cells, but it must be stressed that cell lineage studies alone cannot establish connections be-

tween ancestry and fate. As with the *Xenopus* (clawed toad) central nervous system⁶, tests of these relationships must wait for experiments in which particular lineages are challenged to produce derivatives normally made by different clones, as has been accomplished in birds with ectopic grafts⁷, in *Drosophila* with cell-autonomous growth-rate mutants⁸ and in the grasshopper⁹, nematode¹⁰ and leech¹¹ with cell-ablation methods. Molecular probes for special developmental programmes, such as those for some sea-urchin embryonic lineages¹² would also be useful. □

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Theoretical physics

Supertwistors and superstrings

from L.P. Hughston

ROGER Penrose's 'twistor' method¹ has been fashionable for about a decade among mathematicians and mathematical physicists as a powerful technique for analysing certain classes of partial differential equations and the manifolds or spaces that are naturally associated in various ways with them. The systems of dif-

ferential equations to which the method is applicable are those arising in relativistic field theories, and thus there is a deep vein of interest in the theory which links these developments with advances in our understanding of elementary particles and gravitation. Although the method was originally devised with space-time model-

led as a classical four-dimensional manifold, it has become increasingly clear that the theory can in several instances be developed in higher-dimensional spaces, and can be set up so as to admit supersymmetric degrees of freedom.

In particular, G.A.J. Sparling, on page 417 of this issue², formulates in simple terms the field equations of no less than five distinct important theories: the classical Einstein gravitational equations; the classical Einstein-Maxwell equations; the bosonic equations of eleven-dimensional supergravity; the curvature and torsional field equations of supergravity in dimension four; and the equations of graded Einstein-Maxwell theory in dimension four.

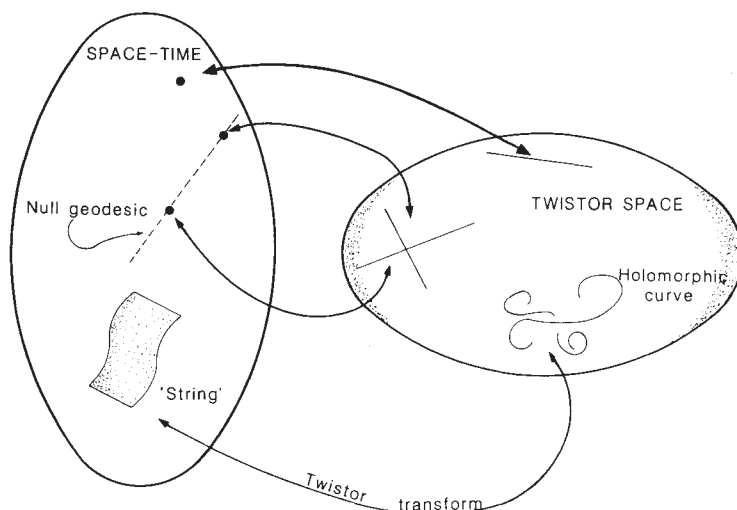
Sparling shows in each case that the relevant field equations correspond to the 'splitting' of a certain bundle structure (the bundle of local twistors or supertwistors). This characterization of field equations by means of a splitting condition is sufficiently compelling that we may view it in effect as providing an alternative to the time-honoured 'principle of least action'.

In his early work on twistor theory³⁻⁵, Penrose showed how insight could be gained into the nature of the geometry of space-time by consideration of a second manifold, twistor space, which is related to space-time via an intricate transformation (see figure). The points of space-time correspond to extended structures (complex projective lines) in the associated twistor space; intersection of a pair of these lines corresponds in space-time to null-separation (that is, connectedness along a light-like trajectory). Penrose and others were able to demonstrate how the elementary linear equations of non-interacting relativistic fields in space-time could be solved, remarkably, by use of essentially 'free' holomorphic data specified on appropriate regions of twistor space.

A significant step forward came about ten years ago with the discovery of the so-called 'non-linear graviton' construction⁶, which showed how certain classes of coupled non-linear partial differential equations could be solved by a kind of non-linear variant of the earlier ideas which had worked for linear theories.

Shortly thereafter it was shown by Atiyah and Ward^{7,8}, Yasskin and Green⁹, Witten¹⁰, and others how, with slight modifications of the basic ideas, theories of the Yang-Mills type could also be treated by twistor methods. Witten¹⁰ played a key part in subsequent developments because, following on from the ideas of Ferber¹¹, he was the first systematically to wed supersymmetry techniques to the geometrical methods of twistor theory.

Since then there has been widespread activity in generalizing the Penrose transform and related ideas to higher-dimensional systems and to systems of interact-



Points in space-time correspond to extended structures (complex projective lines) in twistor space. Space-time points which are null-separated correspond to lines which intersect. A relativistic string corresponds, in twistor space, to a type of holomorphic curve.

ing fields. Witten, in particular, recently devised a supersymmetric twistor-like transform in ten dimensions¹², motivated by the possibility that such a transform may be "the proper starting point for understanding the geometrical meaning of superstring theory".

Sparling, following to some extent the prototype of C. R. LeBrun¹³, now extends these investigations along a promising new line of enquiry, asking, via consideration of the geometry of twistor bundles, for a general characterization of the most significant field equations of relativistic physics.

As for the future, as both Witten and Sparling point out, an important line of development may be to establish further the links between superstring theory and twistor theory. In this connection another recent step forward is relevant: W.T. Shaw has shown¹⁴ that the dynamical equations of classical string theory in four dimensions can be solved by means of a twistor transform. According to Shaw's scheme a string corresponds in twistor

space to a type of holomorphic curve, specified essentially arbitrarily (see figure). Again here the physical object (the relativistic string) is described in terms of free data on the twistor space — the challenge which follows is that of constructing a consistent quantum theory from this essentially classical picture. □

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Molecular pharmacology

Structure and function of acetylcholine-receptor ion channels

from David Colquhoun

FOR much of this century pharmacologists have been saying that once they knew the structure of the cellular receptor, as well as that of the agonists and antagonists that combine with it, then it would be possible to understand the mechanisms of action of neurotransmitters, drugs and hormones. This dream is now being realized, thanks in no small part to the beautiful work of a group at Kyoto University on structure and of a group at the Max-Planck Institute, Göttingen on function of the acetylcholine receptor. In the latest work from these groups, reported elsewhere in this issue (Mishina, M. *et al.* *Nature* **321**, 406; 1986), Numa, Sakmann and their colleagues elucidate the structural basis of the difference between the fetal and adult forms of the acetylcholine receptor in bovine muscle.

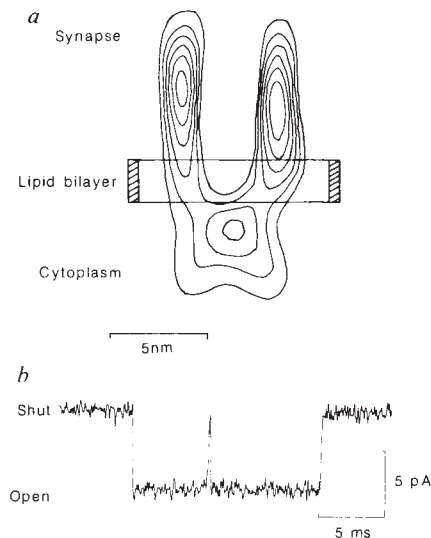
The nicotinic acetylcholine receptor from electric fish is a pentameric protein, $\alpha\beta\gamma\delta$, made from four types of subunit which are arranged around a central ion channel; that from mammalian muscle is almost certainly similar. The primary sequences of all four subunits have now been found using recombinant DNA methods. The subunits span the cell membrane (*a* in the figure); when two acetylcholine molecules combine with sites on the extracellular parts of the α subunits, the ion channel opens for a millisecond or so, resulting in chemical synaptic trans-

mission from nerve to muscle fibre of such rapidity and reliability that it allows us (well, a few of us) to play the Waldstein sonata.

Before the motor nerve reaches embryonic muscle fibres, the acetylcholine receptors are widely distributed over the whole surface of the developing myotube, but following innervation some remarkable changes take place (for a review see Dennis, M.J. *A. Rev. Neurosci.* **4**, 43; 1981). Around the time of birth (in the rat) the rapid turnover of the fetal acetylcholine receptors is slowed, the receptors become highly concentrated over a very small area of the muscle fibre immediately at the junction with the motor nerve ending, and the density of extrajunctional receptors becomes much lower. During the immediate post-natal period the characteristics of the acetylcholine-receptor ion channel change from the fetal to the adult form: the mean duration of an individual opening of the ion channel induced by acetylcholine is shorter by a factor of 3 or 4, and the conductance of the open state is about 50 per cent larger in the adult relative to the fetal form. It is thought that these changes are brought about by some substance released by the motor nerve and/or muscular activity. When the motor nerve of an adult fibre is cut, receptors reappear throughout the length of the muscle fibre membrane, and these new

extrajunctional channels are remarkably similar to the fetal receptors in their turnover time, single-channel conductance properties and mean open lifetime.

There has been much discussion about whether the change from fetal to adult receptor during development results from modification of existing fetal-type receptors, for example by phosphorylation, or from synthesis and insertion into the membranes of new adult-type molecules. Some work has suggested that the turnover rate (and rate of insertion of new receptors) is too slow for the latter mechanisms to be correct. But the new work of Mishina *et al.* shows with beautiful clarity that the adult and the fetal receptors are different molecules. The adult protein contains, in place of the γ subunit found in the fetal protein, the recently discovered ϵ subunit which has a different pri-



Static structure and rapid function. *a*, Electron image map of a cross section of the receptor-ion channel, perpendicular to the plane of the cell membrane; the wide mouth of the channel is clearly resolved (redrawn from Brisson, A. & Unwin, P.N.T. *Nature* **315**, 474; 1985). *b*, A single activation of the ion channel by acetylcholine causes rapid transition to the open state (downward deflection); this channel opening lasts for about 14 ms but the current is interrupted by a brief closure that lasts for about 100 μ s (from Colquhoun, D. & Sakmann, B. *J. Physiol., Lond.* **369**, 501; 1985).

mary sequence (Takai, T. *et al.* *Nature* **315**, 761; 1985). The ϵ subunit has rather more acidic, and fewer basic, amino acids than the γ subunit and it has only 53 per cent homology with the γ -subunit sequence (although this is greater than its homology with the α , β or δ sequences). Furthermore the messenger (m)RNA encoding the γ subunit is found mainly at the early fetal stage whereas the mRNA for the ϵ subunit becomes predominant after birth (contrary to the report of Takai *et al.*) A particularly nice feature of the new work is the direct com-

parison between the functional properties of the receptors (made in oocytes following injection of mRNAs encoding the pure subunits) and the receptors in their natural environment in fetal and adult muscle membranes. The properties are astonishingly similar — perhaps, after all, the environment of the receptor is not very important.

It now seems likely that during development the motor nerve somehow switches on expression of the gene encoding the ϵ subunit while stopping expression of the gene encoding the γ subunit. A few problems remain: it is just possible that the nerve might control expression of an enzyme that selectively breaks down the mRNA encoding one type of subunit; and it is conceivable that the γ subunit of an existing fetal-type receptor could be exchanged for an ϵ subunit while it is in the membrane, although neither of these possibilities seems very likely. There is some evidence to suggest that more than two sorts of channel can exist during development and after denervation. Perhaps this is why the variability of the mean open time of the channel for the fetal type receptor in oocytes is much greater than that of the adult type.

The work by Mishina *et al.* greatly improves our understanding of the relationship between structure and function at the relatively gross level of distinct receptor subtypes. Next, we would like to elucidate the molecular behaviour of a channel when an agonist binds to it. What are the structures of the 'discrete' states inferred from kinetic analysis — for example, what are the structures of the open and shut states; why is it that some agonists can hold the channel open for longer than others; and what is the structural explanation of the brief interruptions in channel openings? These events occur on a time-scale of milliseconds or microseconds, whereas our picture of structure is still static (see figure).

Parts of the receptor molecule have already been changed by site-directed mutagenesis (Mishina, M. *et al.* *Nature* **313**, 364; 1985) and the function of subunit hybrids examined (Sakmann, B. *et al.* *Nature* **318**, 538; 1985). But clearly the substitution of one amino acid in the sequence could change the conformation of the whole subunit, or even that of adjacent subunits. Attempts to assign discrete functions to parts of the molecule will inevitably be ambiguous unless new methods of assessing the higher-order structure can be devised. Judging by experience with other molecules such as haemoglobin, it could be some time before clear answers emerge — but at least now the light is visible over the horizon. □

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Ocean Drilling Program

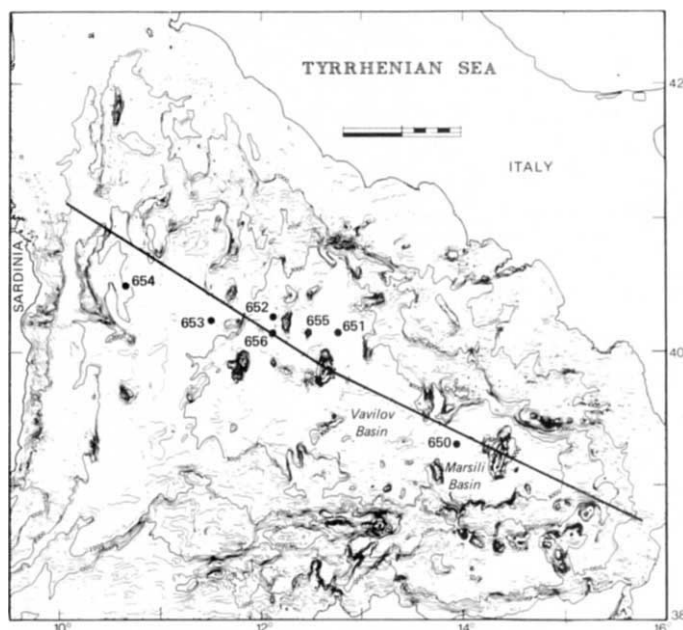
A microcosm of ocean basin evolution in the Mediterranean

from the Leg 107 shipboard scientific party*

THE Tyrrhenian is a small triangular sea surrounded by mainland Italy, Sicily, Sardinia and Corsica (Fig. 1). Leg 107 of the Ocean Drilling Program was the third scientific drilling study in the Mediterranean Sea, following the pioneering Deep Sea Drilling Project legs 13 and 42A in 1970 and 1975. The Leg 107 cruise had three main objectives: to consider the Tyrrhenian Sea as a back-arc basin; as a

lar (full of gas bubbles) which implies that the basalt was erupted into water much shallower than its present 4,100-m depth below sea level. Thus, the eastern part of the Tyrrhenian Sea has subsided markedly since it was formed. At site 655, further north-west, Leg 107 recovered 110 m of basalt flows overlain by sediments that are about 3.5 million years old, and at site 651 the basement is unexpectedly complex —

Fig. 1 Tyrrhenian Sea. Leg 107 drilled a north-west/south-east transect of seven sites (650–656) from the Sardinian passive margin (left) across two small deep oceanic basins. (Bathymetry after International Bathymetric chart of the Mediterranean; contour interval, 200 m; scale bar, 100 km).



young passive margin; and as a stratigraphic type locality.

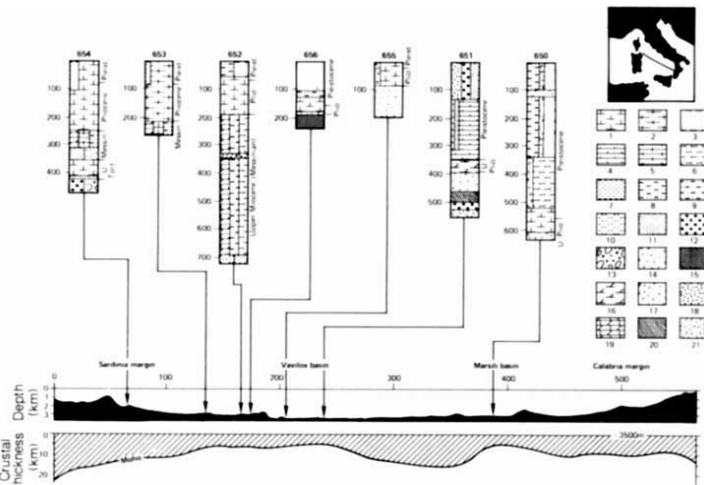
The Tyrrhenian Sea is a small oceanic back-arc basin that has opened behind a volcanic arc and above a subduction zone. Widespread volcanism and frequent earthquakes indicate that the subduction plate boundary is still active and that the back-arc basin is still evolving. Leg 107 investigated how such a basin evolves in space and time. At the south-easternmost drill site (site 650; Fig. 2), we found evidence that the basin is geologically very young (less than 2 million years old). The basalt recovered at site 650 is very vesicu-

lar (full of gas bubbles) which implies that the basalt was erupted into water much shallower than its present 4,100-m depth below sea level. Thus, the eastern part of the Tyrrhenian Sea has subsided markedly since it was formed. At site 655, further north-west, Leg 107 recovered 110 m of basalt flows overlain by sediments that are about 3.5 million years old, and at site 651 the basement is unexpectedly complex —

a cap of basalt flows overlies peridotite, a rock type normally associated with the Earth's upper mantle. Sampling the igneous basement and dating the overlying sediment at sites 650, 651 and 655 shows that the locus of oceanic accretion shifted through time from north-west to south-east, that is, towards the subduction zone. The western Tyrrhenian Sea is a young, passive continental margin that was formed by stretching, faulting and subsidence of the continental crust. At site 654, we recovered a textbook example of a sequence of rock types that indicates a progressive transition through time from a continental to a marine environment. The rocks recovered from the bottom to the top of the hole are subaerial conglomerates overlain first by sandstone with oyster shells and then by open marine sediments. Subaerial, shallow-water and then deep-water environments ensue as the site sank relative to sea level. Lower on the continental margin, at sites 652 and 656, we again encountered evidence of significant subsidence within the oldest sediments drilled that appear to have been deposited

*Co-Chief Scientists, Kim Kastens (Lamont-Doherty Geological Observatory) and Jean Mascle (Univ. Pierre et Marie Curie). ODP Staff Scientist, Christian Aroux (Texas A&M Univ.). Also Enrico Bonatti (Lamont-Doherty Geological Observatory), Christina Broglia (Lamont-Doherty Geological Observatory), James Channel (Univ. Florida), Pietro Curzi (Ist. di Geologia Marina Bologna), Kay Christian Emeis (Texas A & M Univ.), George Glatton (Univ. de Provence), Shiro Hasegawa (Tohoku Univ.), Werner Hieke (Technische Univ. München), Georges Mascle (Univ. de Grenoble), Floyd McCoy (Lamont-Doherty Geological Observatory), Judith McKenzie (Univ. Florida), James Mendelson (MIT), Carla Muller (Univ. Frankfurt/Main), Jean-Pierre Rehault (Univ. Pierre et Marie Curie), Alastair Robertson (Univ. Edinburgh), Renzo Sartori (Ist. di Geologia Marina), Rodolfo Sprovieri (Ist. di Geologia, Palermo), Massimo Torii (Kyoto Univ.).

Fig. 2 Simplified lithological columns for each site are projected onto a bathymetric and crustal thickness cross-section of the Tyrrhenian Sea (cross-section after Steinmetz, L., Ferrucci, F., Hirn, A., Morelli, C. & Nicolich, R. *Geophys. Res. Lett.* **10**, 428; 1983). The position of the transect is shown in the insert. Symbols for lithological columns of sites 650–656: (1) nannofossil ooze; (2) nanno-foram or foram-nanno ooze; (3) calcareous ooze; (4) nannofossil chert; (5) nanno-foram or foram-nanno chalk; (6) calcareous chalk; (7) clay/claystone; (8) mud/mudstone; (9) sandy mud/sandy mudstone; (10) silt-siltstone; (11) sand/sandstone; (12) gravel; (13) conglomerate; (14) basic igneous; (15) pebbles and red carbonate mud matrix; (16) dolomite; (17) volcanic ash; (18) volcanic lapilli; (19) gypsum; (20) complex transition zone; (21) peridotite.



in a lake. Slumps, debris flows and small faults are common in the sediments deposited during subsidence and stretching of the crust. Subsidence seems to have occurred 1–2 million years later in the south-east (site 652) than further west (site 654).

The geological stages for the past 5 million years were all originally defined on the basis of outcrops around the Tyrrhenian Sea. These stages are now widely used outside the Mediterranean, but some doubt remains about the calibration between the boundaries of stratigraphic zones recognized in the Mediterranean Sea and the biozones of open oceans such as the Atlantic and Pacific oceans or Caribbean Sea. Thus, the third goal of Leg 107 was to recover nearly continuous sediment successions and to calibrate the various techniques used to date marine sediments. Such techniques are based on

microfossils, reversals of the Earth's magnetic field, correlations of volcanic ash layers and ratios of stable isotopes contained in the sediments. Drilling at site 653, where we have successfully obtained two nearly continuous sequences of open marine sediments down to 5 million years old, should be suitable for this purpose.

Leg 107 has touched on many other geological subjects, including evidence for crustal heterogeneity, protrusion of upper mantle material, the chronology of volcanic ash layers, cyclic deposition of Messinian evaporites, the origin of organic-rich and metalliferous sediments, the definition of biostratigraphic boundaries and variation of heat flux through the sediment column. The Tyrrhenian Sea is clearly one of the best places for the study of many geological processes. Other areas of the Mediterranean Sea appear to hold similar promise. □

Parasitology

Spurious *Babesia* antigens

from F. E. G. Cox

A PROMISING approach to the development of a vaccine that would protect cattle against babesiosis has suffered a serious setback with the discovery that what seemed to be the dominant proteins recognized by the serum of babesiosis-infected animals merely reflect a host response to an inflammatory reaction¹. Worse still, this discovery is a warning to parallel programmes of vaccine development against other parasitic diseases.

Babesiosis is an important tick-borne protozoal disease of erythrocytes in cattle, affecting several hundred million animals worldwide. Routine immunization can be achieved by vaccination with attenuated

strains of the parasite² and there is also evidence that fractionated parasites can confer some protection³. The search for more satisfactory vaccines is currently following the procedures pioneered for malaria parasites — the identification of major antigens by immunoblotting techniques using immune sera, the isolation of the relevant messenger RNA, transcription to DNA and eventual cloning⁴. This kind of approach has attracted considerable attention and Australian scientists at the CSIRO laboratories at Indooroopilly in Queensland have over the past few years identified several antigens associated with *Babesia bovis*-infected erythro-

cytes that they now believe may be of host rather than parasite origin¹.

Calves with no history of babesiosis and with no detectable antibodies against the parasite were injected with turpentine to induce an acute inflammatory response. At the same time sera were collected from cattle immune to *B. bovis*. Nitrocellulose blots of *B. bovis* antigens treated with anti-babesial antisera from immune animals give rise to numerous reaction bands. The dominant bands revealed were of relative molecular mass 120,000–240,000 and were absent in similar antigen blots treated with control sera. But a similar test with sera from the turpentine-treated animals revealed the same bands. Precautions were taken to ensure that these reactions were not artefacts and the authors conclude that what are apparently *B. bovis* antigens can be detected by sera from animals experiencing a non-specific inflammatory reaction.

The conclusion from these experiments is that these dominant antigens recognized by specific immune sera cannot be of babesial origin, and furthermore that differences in their size range is consistent with their being isoantigens in genetically diverse cattle. Alternatively, these results may indicate interactions between babesias and naturally occurring autoantibodies. The authors suggest that, at the height of parasitaemia, the inflammatory reaction results in the release of acute-phase proteins simultaneously with the depression of the immune response to parasite antigens. Thus, the antigens recognized at this stage are those associated with inflammation and not infection.

These results also explain earlier findings that sera from uninfected cattle sometimes produce a positive immunofluorescence in the indirect fluorescence antibody test in which both *B. bovis*-infected erythrocytes and the parasites themselves fluoresce⁵. Overall, the significance of these findings is twofold. First, they pinpoint possible errors in immunodiagnosis; second, and most important, they suggest that immunoblotting techniques may identify spurious candidate antigens for the possible development of vaccines. This warning must be heeded in fields other than babesiosis for there have been reports that sera from uninfected animals react with various antigens of protozoan and helminth origin⁶. □

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Predicting that comet Halley is dark

SIR—As I write this letter in early March only the scantiest information has come out of the Giotto mission. One of the most interesting preliminary findings was that comet Halley appeared to be quite dark. This may seem to be inconsistent with the high degree of reflectivity one might expect from an icy comet nucleus¹, implying that something extra is required to blacken the surface. I should like to show that a very low reflectivity is a predicted² and natural consequence of the comet nucleus being a fluffy aggregate of small solid interstellar particles² each of which is largely water ice and at least moderately reflecting. The reflectivity of the comet is low because it is an open structure and light which penetrates it is internally absorbed by being scattered many times by the individual small particles which are its components.

The first stage of interstellar particle coagulation in the presolar nebula which is assumed to lead ultimately to the comet nucleus is shown schematically in Fig. 1 by a model containing 91 individual particles. Each particle is a mean representative of a size distribution of interstellar grains which have fully accreted all the condensable molecules from the dense molecular cloud out of which the solar system was born. It consists of a silicate core, an inner mantle of complex organic refractory material which has evolved by ultraviolet photoprocessing over hundreds of millions of years before comet coagulation, and an outer mantle of volatile ices in which H₂O is dominant³ and which contains S₂ molecules which could only have been produced in the interstellar dust before coagulation⁴. These particles are typically ~0.5 µm thick and at least three times as long⁵. Imbedded in the outer mantle are many small (~0.01 µm radius) particles (some strongly absorbing) which show up in space as blocking the ultraviolet radiation of stars⁶.

The porosity shown in Fig. 1 was derived by comparing the density of comet dust with the mean density of meteors which are known to be comet debris. Although the mean density of the non-volatile silicate core-organic refractory mantle interstellar grains (no outer icy mantle) is about 1.9 g cm⁻³, a characteristic mean meteor density of 0.2 g cm⁻³ is observed⁷. This obviously implies a high degree of porosity. The organic refractory component is the largest part of the comet dust after the volatiles evaporate. Taking into account the volume of volatile ices in the precometary dust (and thus in the original comet matter) one arrives² at a mean packing factor for the comet of no more than about 0.4 (60% empty space). This is what has been pictured for the 4

µm aggregate in Fig. 1. An even fluffier comet nucleus structure is probable since the meteor particles must undergo some collapse after evaporation of the volatile components⁸.

It is therefore reasonable to approximate the light scattering by a comet as if it were a cloud of small particles each of which is characterized by an albedo α and a scattering asymmetry factor g . The individual particle albedo is defined as the ratio of the light scattered by the particle to the light it both absorbs and scatters (no absorption: $\alpha = 1$; total absorption: $\alpha = 0$). The asymmetry factor, defined as the mean value of the cosine of the scattering angle, is essentially the difference between the forward scattered and the backward scattered light (spherically symmetric scattering: $g = 0$; no back scattering: $g = 1$). At visual wavelengths the normal interstellar grains (no icy coating) have an albedo $\alpha = 0.6 \pm 0.1$ and an asymmetry factor $g = 0.8 \pm 0.1$ (ref. 9). It is a well known property of the scattering of light by small particles that in the size to wavelength range of interstellar dust, a further increase of particle size by the addition of icy mantles leads to an increase in the asymmetry factor up to $g = 0.9$ (ref. 10). The very strongly forward directed scattering implies that light impinging on a particle at the comet surface mostly continues inward where it may be absorbed by repeated scatterings within the body.

Let the forward and backward scattering fractions be F and B so that $F + B = 1$. Using $g = F - B$ the backward scattered fraction is $B = (1-g)/2$. Since only a part, α , of the incident radiation per particle is scattered and, since we shall assume that all the forward scattered radiation is lost, the net back scattering by the sum of the individual particles filling the surface of the comet is the total reflected light. This gives a reflectivity of $A = \alpha(1-g)/2$. So

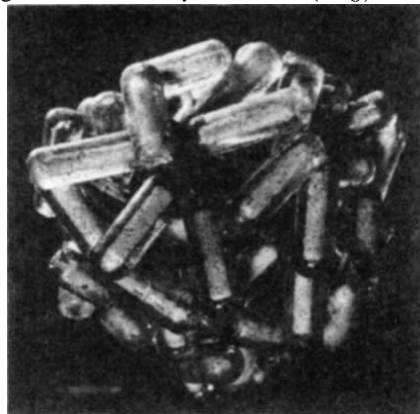


Fig. 1 A model of randomly coagulated core-mantle dust grains. Shown is an ensemble of 91 particles incorporating within their outer icy mantles the very small particles of the interstellar dust population accreted in the final stages of condensation. The degree of volumetric packing is 0.4 (60% open space) and the overall dimension is scaled to about 4 µm.

for $\alpha = 0.6$ and $g = 0.9$ only about 3% of the light is reflected. This simple but physically reasonable approximation for the reflectivity matches remarkably closely the value $A = 0.028$ obtained from very accurate calculations of Van De Hulst¹¹.

For the same reason, large comet dust particles should similarly exhibit low reflectivities, as may have been detected in comet Crommelin¹². Judging by the high infrared emissivity of interplanetary particles well beyond the Earth's orbit (S.S. Hong, personal communication) they too, as young comet debris, are highly absorbing because of their fluffy character.

Other critical predictions of this model of the nucleus are: (1) A preponderance of the organics C, O, N relative to the rocky elements Si, Mg, Fe by a factor of five or greater in comet dust grains because of the initially high ratio of the organic refractory to silicate volume in the interstellar core-mantle grains². (2) Individual core-mantle particles of order 0.15 µm radius and mass about 3×10^{-14} g resulting from aggregate breakup. (3) A very large number of very small (~0.01 µm) particles of mass of about 2×10^{-17} g released from the evaporating icy material¹³. (4) A very low comet heat conductivity leading to high surface heating and a concomitant high vaporization rate due to the very porous surface area.

Should the Giotto and Vega missions confirm these predictions we may well be able to say that comets provide a direct observation of the interstellar dust as it existed 4.5×10^9 years ago and that a comet-nucleus-sample-return mission will accomplish what no amount of remote sensing of the interstellar medium will ever make possible.

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Multiple sclerosis, viruses and glycolipids

SIR—For many years now, and again recently in *Nature* (318, November 1985 p101, p104, p154), there has been speculation as to the role of viruses in multiple sclerosis (MS). MS is often considered to be an autoimmune disease initiated by infection; the question has always been and remains, which of the many viruses implicated causes the disease? One possibility, which has perhaps not received sufficient attention, is that MS could be caused not by just one virus but by many enveloped viruses acting together or individually.

All the viruses that have been implicated in MS are capable of infecting the nervous system, and all are enveloped viruses. The membrane of an enveloped virus reflects that of the cell from which it has budded. Indeed it has been suggested that such viruses may be able to trigger autoimmune reactions by the incorporation of host protein into their viral envelope but, with a few possible exceptions, the budding virus takes only the virally-coded proteins which have been inserted into the cell membrane, and the lipid molecules surrounding them. It is the host-cell derived lipid molecules that could be the key to the problem.

The lipid molecules of cell membranes have suffered for too long by being represented as the homogeneous tadpole-like molecules of the cell membrane. In fact they are far from being homogeneous in either their biochemistry or their distribution. Neither are these molecules immunologically inert, they are haptens and can under the right circumstances induce an immune response.

In recent years it has been shown that antibodies to glycolipids are detectable in MS patients¹, that such antibodies can demyelinate cerebellar tissue cultures², that T-cell reactivity to glycolipids is present in MS patients³, and relatively specific to this disease⁴, and that rabbits inoculated with glycolipids succumb to a demyelinating encephalomyelitis⁵. Thus it is clear that anti-glycolipid activity is of importance in demyelinating disease.

The question is, how does this anti-glycolipid activity arise? One possibility, as we have suggested previously⁶, centres on the possibility that budding viruses can initiate an immune response to glycolipids. We have shown that infection of mice with the budding, avirulent RNA, Semliki Forest virus induces a T-cell dependent demyelinating encephalomyelitis⁷, although it is not yet clear whether the T-cells responsible for the demyelination react against viral or self antigens on the surface of central nervous system (CNS) cells and myelin. The virus replicates in CNS cells including oligodendrocytes, host-cell glycolipids are incorporated into the viral envelope⁸, and these

glycolipids are accessible to antibodies⁹. Infection with the virus and inoculation of inactivated brain derived virus both give rise to anti-glycolipid antibody (S. Amor and H.E.W., unpublished observations).

It is possible that several neurotropic budding viruses have the ability to replicate in the same CNS cell-type (for example oligodendrocytes) and on budding from this cell incorporate into their viral envelope the same host-cell glycolipid (for example galactocerebroside). It is possible that such a glycolipid (normally a hapten), associated on the surface of the virus with the 'foreign' carrier antigens of the viral proteins could be antigenic. In an MS susceptible individual, infection of the CNS with one of the many possible enveloped viruses could trigger an anti-glycolipid immune reaction, leading to demyelination and a first attack of MS. Subsequent relapses in MS, already known to have an association with inter-current infection, could result from re-stimulation of the same anti-glycolipid autoimmune response following CNS infection with other enveloped viruses.

Perhaps in the search for the cause of MS, more attention should be given to the possibility that viral envelope glycolipids could induce an immune mediated demyelination, and by the immunologists to the possibility that this may trigger an autoimmune encephalomyelitis.

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Ventral activation process in insect oocytes

SIR—In a recent *News and Views* article Woodland and Jones reviewed genetic evidence of an active ventral region in establishing the dorsoventral polarity and hence pattern in *Drosophila* eggs¹. In particular, various recessive — and presumably loss of function — genes are all dorsalizing: not only the genes so lucidly discussed by Woodland and Jones but also the remarkable *K10* gene. Unlike the other dorsal mutants, *K10* acts on the growing oocyte so as to largely symmetrize the early movements of its follicle cells. Thus it produces an egg largely devoid of dorsoventral asymmetry in its

shape and shell as well as dorsalizing its embryo^{2,3}. Thus lack of *K10* function seems to disable the mechanisms for establishing a dorsoventral gradient. Since it does so by disabling some ventrally localized process, it acutely raises the question of just what this ventral process is.

A clue to this was provided by Kunkel in a study of an analogous growth stage of the cockroach oocyte⁴. He has discovered the presence of large, steady electrical (and thus ionic) currents entering its ventral region. Comparable steady currents rather generally enter regions of developmental action, and in the best studied case — that of the fucoid egg — they seem to establish this region by raising free calcium there^{5,6}. So Kunkel's observations suggest that a key early event in *Drosophila* pattern formation is an influx of cations into its ventral region which raises free calcium there, activating gene products like those of *Toll*.

This hypothesis also suggests an explanation for the rather puzzling fact that *Toll*⁺ cytoplasm from *anywhere* in the egg nevertheless induces a ventral region near the site of injection. Perhaps the *Toll*⁺ precursor postulated by Anderson *et al.*⁷ is artificially activated *during transfer* by contact with the high calcium found in the perivitelline fluid of the *Drosophila* egg⁸. This subsidiary hypothesis predicts that the ability of *Toll*⁺ cytoplasm to rescue *Toll*⁻ eggs would be greatly reduced if it were transferred in a way which somehow avoided any transient rise in its free calcium concentration. This postulated artefactual rise would be analogous to the rise in calcium which can destroy the so-called primary cytoskeletal factor in the cytoplasm of frog eggs⁹.

In any case, the main hypothesis predicts the presence of a free calcium gradient across the dorso-ventral axis of the growing insect oocyte with free calcium high along its ventral face. This predicted ventral high calcium zone would be analogous to that already seen — with the aid of aequorin — at the vegetal pole of the early medaka fish egg¹⁰.

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The hungry sheep look up

Walter Gratzer

How to Obtain Biomedical Research Funding. Edited by J.T. Dingle.
Elsevier Applied Science: 1986. Pp.56. Pbk £5.95, \$10.

A NEW cottage industry appears to have established itself around the sleazier fringes of science in America, and I take it for an omen of the times: announcements have been appearing on notice-boards around the country proclaiming one- and two-day seminars (\$200 a throw seems about average) on how to write successful grant applications. Now it must surely occur to all takers that the performers at these events have entered the business of explaining to other people how to get grants only because they have not lately managed it themselves. They are bald men selling hair-restorer. Successful scientists will in general be too busy insuring against failure the next time round. And besides, as Logan Pearsall Smith observed, "few things are more shocking to those who practice the arts of success than the frank description of those arts".

So what have we here? This appears to be the first guide to working the British grant market, and let me quickly add that I have no doubt the three highly respected scientists, who have contributed to this slender paperback (4 mm in its covers), all have abundant grant support, and no idle half-hours to fill for pecuniary gain. What then has motivated their endeavour? Not, for the most part, any unusual luminosity of insight. Slim this volume may be, but there are still indications of a certain desperation in the efforts to give value. The kind of advice that the editor, Dr J.T. Dingle, dispenses is too often along the lines of "... it must be concise, to the point and interesting". How so? Why, doctor, because "a dull and repetitive introduction, a turgid summary and incomprehensible experimental detail will not improve the chances of even the most original idea being accepted". Four pages on we are enjoined to "try to be concise and organise one's arguments in short but cohesive sentences", and on the next page, "the summary should state precisely and clearly the objectives of the study". Well, you could have knocked me down with an RG2A application form!

Dr J.L. Gordon from the Clinical Research Centre weighs in with a chapter about the referee's view of grant applications. "What", he asks, "determines whether a referee will agree to review an application?". And he has the answer: "he has to ask himself two questions: first, is this my field? And secondly ..."; but there, I shall not spoil the impact of the

narrative by giving away any more. You must buy the book.

Matters look up with the eruption onto the scene of Professor Jack Lucy, who gives a wholly convincing and distinctly diverting account of how the deliberations of a Medical Research Council Grant Committee might go, confronted with a typical (though imaginary) application. Here are two of the eleven evaluations by the committee members. Number 7 opines that

The applicant has worked in this field for many years but of late there have been diminishing returns. It seems highly likely, unless a new approach is developed, that there is little prospect of this research yielding any observations of significance, and it is suggested that the applicant might be advised that the most likely way of obtaining a further insight into this problem is by gene cloning.

And Number 8:

The applicant has a good record of many years of research in this field but now, quite correctly, judges that — if much further progress is to be made in this area — the newer techniques of recombinant DNA technology and site-directed mutagenesis need to be employed. Unfortunately, the applicant has no experience at all in the use of these techniques ... I there-

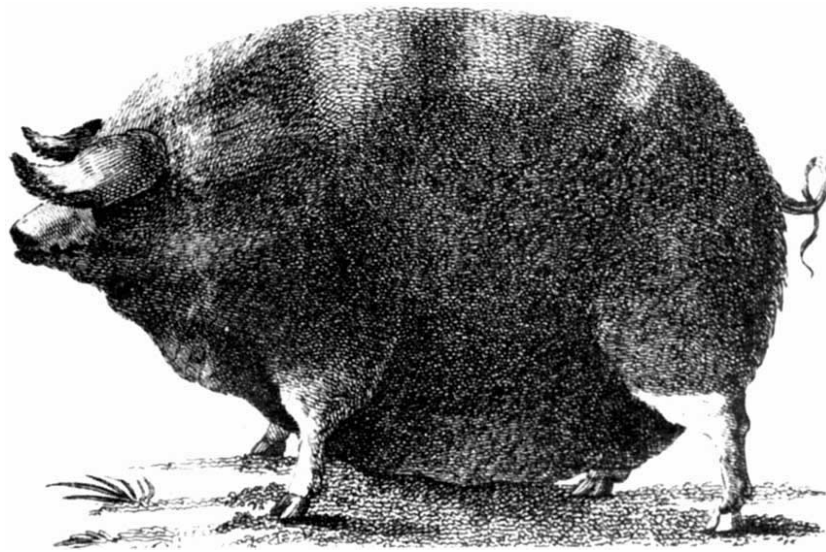
fore have serious reservations regarding the viability of this otherwise potentially-interesting project.

The ring of truth is unmistakable. This is much more the kind of thing one is looking for.

The last twenty pages of the book are by administrators, and there are no laughs but lots of information. They explain how the MRC and the private charities operate their grant systems, and an appendix lists eighty organizations that will disburse the moola when appropriately stimulated.

This small book then could with advantage have been even smaller, but it will have its uses. One question lingers in the mind: allow for the moment that such a publication as this might contain the key that will unlock the strong-room. Will it then work for the common weal? The Law of Conservation of Matter must surely operate: what each purchaser of the book collects will be snatched from you and me, and have we not all seen the undeserving flourish, yea, as the green bay tree in fact? I would incline to the view that those who cannot work out for themselves that the summary should be clear, concise and fit into the box provided, would do better selling stock in biotechnology, or even explaining to anxious young scientists How to Obtain Biomedical Research Funding. □

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Fat pig — the illustration, from an agricultural survey of Staffordshire in 1796, is of an animal that was 802lb liveweight at the age of 2½ years. It was by no means the largest recorded. The tendency towards breeding pigs that became excessively fat at a relatively young age began in the late eighteenth century with the introduction of early-maturing foreign breeds to Britain. Because of the demands of the show-ring and the lard industry, the problem became progressively worse through the nineteenth century: one judge at the Royal Show in 1881 felt obliged to comment that "it is painful to see the prostrate masses of fat grunting and sweating under a weary life in the heat". Such opinions went unheeded, many breeds became extinct and the British pig industry suffered accordingly. The picture and historical details are taken from Julian Wiseman's entertaining and informative book *A History of the British Pig*, newly published by Duckworth. Price is £12.95.

Crystallographers' proteins

Kenneth C. Holmes

Diffraction Methods for Biological Macromolecules, Parts A and B. Methods in Enzymology Vols 114 and 115. Edited by Harold W. Wyckoff, C.H.W. Hirs and Serge N. Timasheff. *Academic: 1985. Part A pp.588, \$70.50, £59. Part B pp.485, \$66.50, £50.50.*

THE structural investigation of protein crystals by X-ray diffraction started in 1934, when J.D. Bernal and Dorothy Crowfoot Hodgkin first successfully photographed a crystal of pepsin. The resulting X-ray diffraction patterns contained so many Bragg reflections that they resisted all known methods of interpretation for 20 years, until Max Perutz was able to show that the small changes in X-ray intensities induced by binding two mercury atoms per molecule of haemoglobin were enough to determine the phase of each reflection. With phases, the electron density can be calculated, albeit by evaluating a Fourier series of around 10,000 terms at each of 100,000 lattice points! Another five years elapsed before John Kendrew's team could carry out a Fourier synthesis for myoglobin to atomic resolution, revealing for the first time the intricacies of the three-dimensional structure of a protein.

Kendrew used programs devised to run on the Cambridge Mathematical Laboratory's EDSAC II. Without such a machine the calculation of the electron density of myoglobin would have been virtually impossible; indeed, the growth of protein crystallography is intimately entwined with the development of the computer.

In the 1950s, because of the influence of Sir Lawrence Bragg and the activities of Perutz and Kendrew, protein crystallography was synonymous with the Medical Research Council unit in the Cavendish Laboratory. (Later, other centres appeared, but even these, in most cases, trace back in apostolic succession to Bernal or Bragg.) In the late 1950s and 1960s, then, a stream of (mostly American) post-doctorate students descended on the MRC unit and worked feverishly to turn protein crystallography into an established science worthy of associate professorships. Now they and their acolytes, and some converts, have written it all down.

The articles in the resulting two volumes are rather technical or mathematical. They are also somewhat uneven in type, ranging from user's guides for various program packages to some thoughtful review papers. Thus, while specialists will find a wealth of useful detail here, those

less familiar with the field could have trouble finding their way around. The contributions are grouped in sections: history (an engrossing article by Perutz); crystallization (the nearest we get to wet biochemistry); data collection; phasing; model building; and presentation of results.

Michael Rossmann, who was in at the start on EDSAC II, contributes a detailed and informative chapter on oscillation photography. Other mandarins also make interesting contributions: Fred Richards concerns himself with the problem of defining protein surfaces and, besides, reviews his folly. David Stuart and David Phillips present an excellent analysis of thermal motion and its relationship to molecular dynamics — otherwise hardly mentioned. Hal Wyckoff, also an editor, has provided a definitive review of diffractometry. Jane Richardson's stylized drawings of proteins are truly beautiful, while Lynn Ten Eyck deserves a prize for the most concise account of X-ray diffraction. A summary of the least-squares

method has been contributed by Robert Sparks, though this is somewhat out of place in the "phasing" section. No less than 12 articles use the least-squares method and the consistent adoption of Sparks's notation throughout the book would have helped clarity.

The article by Joel Sussman on constrained least-squares refinement re-awakened my memories of 1963, when Carl Branden and myself battled with an IBM 7090 for 16 hours to achieve one cycle of constrained refinement of myoglobin. Progress indeed! Once or twice elsewhere I experienced *déjà vu*; for example, mirror benders turn up attributed to all and sundry, but not to Tony Woollard who actually developed the present design in Cambridge. This reflects the fact that in the heady years of the 1960s the rate of innovation outstripped the rate of publication. The present volumes redress the balance with a little to spare. □

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The common quest

A.R. Rees

Growth Factors and Transformation. Edited by James Feramisco, Brad Ozanne and Charles Stiles. *Cold Spring Harbor Laboratory: 1985. Pp.450. Pbk \$70.*

In its application to the study of oncogenic transformation, the inductive approach to science has perhaps come under closer scrutiny than in any other area of biology. While it is generally agreed that cancer is not a single disease, this has not prevented researchers from assuming that common mechanisms will emerge if we just look long enough and hard enough. This is by no means obvious. For example, that tyrosine phosphorylation is a causal event in transformation was and remains an attractive hypothesis; but there is still not a single example in which specific substrates of tyrosine kinases can be said to play a key role in the transformation process, although the presence of the kinase itself is often sufficient to transform the target cell. Even if plausible mechanisms for oncogenic transformation were to emerge, we are still left with the problem of how they would relate to transformation *in vivo* — despite the fact that some 30 oncogenes have been characterized that originate within the cell and 10 or so others from within oncogenic viruses.

It was against this background that, in the autumn of 1984, Cold Spring Harbor Laboratory organized its meeting on growth factors and transformation. The subject matter in the resulting book is arranged loosely into chapters, the first five

of which survey the properties of growth factors (epidermal growth factor, transforming growth factors, insulin and the related insulin-like growth factors, platelet-derived growth factor and its oncogenic relatives, and, finally, the lymphokines). The second half of the proceedings is devoted to oncogenes and kinases, with a concluding chapter entitled "Futures". In the main each author provides an integrated account of the interplay between growth factors and oncogenes, but the existence of genes for which a growth factor connection (or in some instances, function) has not been established necessitated their collection into the separate chapter on oncogenes. For a number of these oncogenes (E1A, Ha-ras-1, c-fos, c-myc, for example) this biological apartheid would still be necessary.

There are always things to be said for and against conference proceedings, even up-market productions such as those emanating from Cold Spring Harbor. In a fast moving field like this, speed is crucial. Unless the proceedings appear within six months they are generally of dubious value to research workers, though they may be useful as background reading for those at the periphery. *Growth Factors and Transformation* appeared in Britain almost a year after the conference. This delay would have mattered less if some of the chapters had not been quite so repetitive. And this criticism might have been redundant if the meeting itself had not been so parochial — only four papers were contributed by groups working wholly outside the United States.

The repetitive element is at one of its high points in the opening chapter on epidermal growth factor, and is also a feature

of the three *c-fos* papers in the "Oncogenes" chapter. For all that, the discussions of the manner in which some transformed epithelial cell lines and their *in vivo* analogues seem genetically predisposed towards either aberrant or over-expression, or both, of EGF receptor transcripts suggest a potentially important correlate of epidermoid malignancy. The more mechanistic aspects of the action of EGF and other growth factors are presented in the "Kinases" chapter. Here the particular role of tyrosine kinases is explored, alone and in their relationship to that possible arbiter of tumour promotion, protein kinase C. It is here also that an organizational eccentricity placed the second of the two related accounts of phosphorylation of 40S ribosomal protein S6, the first being in the opening chapter on EGF.

If we are to take the "Futures" title of the concluding chapter literally, then we are faced with the prospect that studies of certain phenomena — Na^+/H^+ exchange, mitochondrial responses to growth factors, and growth factor effects in systems such as embryonal carcinoma and the developing nervous system — are the subjects of the future. In fact the manner in which growth control signals are developmentally regulated is an undeniably important area of research. But with the exception of the first paper in this chapter (which explores the thesis that "in the future it is likely that the level of biochemistry of cell proliferation, not as well developed now, will become ever more important"), the conclusion is a rather mixed bag of topics that the organizers obviously could not easily fit in elsewhere.

Many of the presentations in this volume are well worth reading, and the book should certainly be in every library pretending to cover the literature on cancer. Whether individuals in the field will find it of much value, I doubt, because most of the data it contains have now been published or even superseded. Non-specialists, however, may find the book a helpful summary of current problems in oncogene research and of the type of experimental approaches that are available to pursue the search for that elusive Holy Grail — the common mechanism. □

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New in paperback

- *The Musical Mind*, by John A. Sloboda. Publisher is Clarendon, price is £9.95, \$17.95. For review see *Nature* 315, 696 (1985).
- *The Computer Comes of Age*, by R. Moreau. Publisher is MIT Press, price is \$9.95, £9.95. For review see *Nature* 312, 572 (1984).
- *The Stars and the Stones: Ancient Art and Astronomy in Ireland*, by Martin Brennan. Publisher is Thames & Hudson, price is £7.95. For review see *Nature* 306, 516 (1983).

Down to the bones

Bernard Wood

The Human Skeleton. By Pat Shipman, Alan Walker and David Bichell. *Harvard University Press*: 1986. Pp.343. \$27.50, £23.50.

THE very word "skeleton" has macabre, if not sinister, associations, and its colloquial use also betrays our perceptions of the human frame; we have all experienced privations in hotels which have been excused by muttered references to the "skeleton" staff. Traditional (by which I mean old-fashioned) medical courses did little to enhance the image. Students were drilled in detailed osteology and every bony lump, bump, nook, cranny and crevice had a name which had to be memorized. Such minutiae provided aggressive examiners with the necessary ammunition to trip up even the most nimble student, but had few other merits for a general undergraduate education in anatomy. It is not surprising that the efforts of physiologists and biochemists to enliven teaching on the skeleton by reference to its metabolic relevance made little headway against this type of introduction.

The image of the skeleton is changing, and this book will help hasten the process. Research on the structure and function of hard tissues, at the levels of cell biology and whole animal studies, are invigorating our attitude to both bone and bones. The authors are particularly well-qualified to provide an applied and broad biological perspective on their subject matter. Pat Shipman has pioneered rigorous methods for assessing the implications of marks on bones found at archaeological sites, while Alan Walker has carried out innovative analyses of primate behaviour and has made important contributions to palaeo-anthropology and palaeopathology. Their colleague, David Bichell, is an artist who is trained in anatomical drawing.

The book is in three sections. The first deals with the components and organization of bone and discusses its physical properties. The central, and largest, section is a series of regionally-based descriptions of the form of the normal skeleton, augmented by succinct, but useful, presentations of integrated activities such as breathing, walking and chewing. The third section covers the ways in which the skeleton, and its components, can be used in forensic and anthropological research. Predictably, the chapter on skeletal pathology is a strong one, and it was sensible to include an account of the structure and identification of teeth.

In terms of scientific content, the authors have wisely eschewed the exotic. One of the book's strengths is that it presents sound skeletal biology in a way

New journals review



On 25 September *Nature* will publish the sixth annual review supplement devoted to science journals.

Criteria for inclusion of a journal in the 1986 issue are that:

- (i) the first number appeared, or the journal was retitled, between June 1984 and May 1985 (the second cut-off date allows at least three issues of a journal to have been published, the minimum number on which a reasonable judgement can be based);
- (ii) it is published at least three times a year;
- (iii) the main language used is English.

Publishers and learned societies are invited to send four different issues of each suitable periodical, including the first and most recent numbers (if from outside the United Kingdom, by air mail) to: The Review Editor, *Nature*, 4 Little Essex Street, London WC2R 3LF, England. Subscription details for both 1986 and 1987 should be included.

that will stimulate students to learn more for themselves. The style is such that quite complex principles and ideas are introduced and discussed without intimidating the reader. There is little doubt that this volume will have a long life, and in any new edition the authors might consider setting the vertebrate skeleton in an even broader context than they have done. Their classification of joints is traditional and, for all its apparent complexities, they might consider making more explicit reference to MacConaill's analysis of joint surfaces.

For those of us who bemoan the demise of Le Gros Clark's *Tissues of the Body*, this new book provides a helpful account of hard tissues and the skeleton. Other textbooks and reference works will be needed to provide extra detail, but few are likely to offer a more stimulating introduction to the subject than that set out by Shipman, Walker and Bichell. □

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Newts and nuclear winters

John H. Lawton

The Machinery of Nature. By Paul R. Ehrlich. Simon & Schuster: 1986. Pp.320. \$18.95.

I MUST confess to breaking the law by catching great-crested newts, lovely, ugly creatures with orange bellies, warty backs and crests like those of a dragon. Under the British Wildlife and Countryside Act, catching great-crested newts even to admire them is now illegal, because they are rare, declining in numbers and endangered. So you need a permit to fish for them, and I didn't know. I do now, and promise to behave myself in future. But it is very sad; as a schoolboy I caught "water dragons" easily and kept them for a time in an old sink. There is nothing like having a dragon in the kitchen for learning about biology. Now it is illegal.

So what? Book reviews do not usually start with confessions of guilt, even of little crimes. There is however a purpose in my confessions, because these muddy little creatures symbolize the problems that confront conservationists as we struggle to hold back mankind's creeping extermination of thousands of endangered species throughout the world. We do not even know how many species of plants and animals there are, nor exactly how many have already been lost through human activities. We do know that the pace of extinctions is rising and that we need to take ever-more drastic steps to stop it. Laws against catching newts are a drop in the ocean compared with the action that needs to be taken. Which brings me logically, if indirectly, to Ehrlich's book.

The Machinery of Nature has several themes. One is an eloquent plea for a halt to the inexorable, blind destruction of the species of plants and animals that are the newt's and our fellow travellers on this planet, because some of them are beautiful and others are useful. But most are neither; we must conserve them simply because they are there, and because we do not know what they do and have only the sketchiest idea about how the machinery of nature really works. Once we have taken the machine to pieces and lost some of the bits, we should not gamble on being able to put it back together again. The biggest immediate threat comes from the growing human population, rising expectations and grubby, creeping degradation of all that is varied and beautiful in nature. But instant, cataclysmic destruction in the inconceivable horror of a nuclear war and the freezing terror of a nuclear winter is also a stark possibility, and is a second, recurring theme of the book. A third is the

excitement and uncertainty of doing science, mixed with a passionate love of natural history — the newts-in-the-kitchen syndrome. Last is a plea for more funding for ecological research. How can we possibly understand the machinery of nature on minuscule budgets that are a direct result of government indifference to environmental science?

The components of the machinery as Ehrlich sees them are the usual mix of population ecology and evolution, socio-biology, "two-species" interactions (predation and so on), biogeography and the dynamics of communities and ecosystems, constrained by the physical environment. However this is no ordinary ecology textbook. What it aims to do is explain the science of ecology to the layman and to our political masters. The intention is fundamentally to change attitudes about what we can and cannot do to the planet and get away with; it is a scientific manifesto for the Green Party, written by a first-class scientist.

The book is certainly well written, although it does have some distracting features. One is Ehrlich's tendency to describe his friends, associates and graduate students as brilliant, and by default mark everybody else beta minus. It is clearly done to enliven and humanize the science; I found it mildly irritating. Also the chapters are too long and sometimes lack a sense of direction; basically they are walks through natural history and the science of ecology, with the path frequently deflected in unexpected, albeit interesting directions. My preference is to know where I am going, and why.

One criticism is more substantial. Ehrlich is American and the book is directed explicitly at his fellow countrymen. Yet the problems it addresses are global and concern everybody. It makes no sense to talk just to Americans, even though (for several reasons) that may be a good place to start.

Will the message have any effect? I hope so, but fear not. It has been preached many times before to little or no avail. For example, by coincidence I recently came across this:

It is very certain that the earth is not the chief body in the material universe, and that the world is not subordinate to man's use. It is even more certain that nature is the expression of a definite order with which nothing interferes, and that the chief business of mankind is to learn that order and govern themselves accordingly.

The writer was Thomas Henry Huxley in 1880.

One hundred years later the message is the same but much more urgent. Whether the majority of mankind understand it well enough to respond is another matter. It takes time and effort even to start to understand the machinery of nature, and most decision-makers and politicians do

not have that time, or care too little to find it. But the problem goes deeper than that. It is one thing to educate lay people about ecology, even assuming that they might be interested. It is quite another to translate that knowledge into a workable political philosophy, and even harder to put it into practice. Indeed, one reason why the great surge of environmental consciousness that swept through the 1960s fizzled out was because it was politically naive. With millions out of work in Europe, still more starving in Africa, grinding poverty, social unrest, terrorism and super-power confrontation, who cares about newts? The problem is getting decision-makers to understand enough of the machinery of nature to realize that we are slowly degrading the life-support systems of the planet, without being able to predict when things might start to go badly wrong (assuming that they haven't already) and to take a longer view about what is important. We also have to translate environmental science into practical, realistic political policies and, in Huxley's words, govern ourselves accordingly. Ehrlich's book is an attempt to educate, but it has nothing to say about welding science to practical politics.

Of course there is general agreement about some issues. Only lunatics want a nuclear war. Yet the body politic is conspicuously unable to reduce, let alone get rid of nuclear weapons. So we pass laws protecting newts, fiddling quietly whilst Rome teeters on the edge of the holocaust. Worse, we do not even take the laws that we have to protect nature as seriously as we should. It is irritating to have to change your behaviour or, worse, abandon a road-building project for the sake of a newt. Yet if rich, developed nations cannot look after their remnants of wild nature, how on Earth can we expect some of the poorest countries in the world to conserve great rain forests, coral reefs or swamps? Who is going to pay to keep the lions wild, and the deserts at bay?

The forces ranged against conservation are formidable. Some of them are evil, some greedy, some both. But most are simply ignorant of the dangers, and too preoccupied with the problems of today to care about the next generation. I do not pretend for a minute that Ehrlich's book will transform the picture overnight, or even over the next decade. But it will help to educate the growing number of people who want to know about the machinery of nature, and about the stark choices that confront mankind between now and the end of the century. Whether you are concerned about newts or nuclear winters, there must be a better way to manage the planet. The price of failure doesn't bear thinking about. □

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Space observations of comet Halley

from John C. Brandt

Following the encounters by Soviet, Japanese and European spacecraft, the International Cometary Explorer took measurements of comet Halley on 22 March. The comet has also been observed by the Solar Maximum Mission, the International Ultraviolet Explorer, the Pioneer Venus Orbiter, the Dynamics Explorer 1, and by sounding rockets.

FOR several days before and after its encounter with comet Giacobini–Zinner on 11 September 1985, the International Cometary Explorer¹ (ICE) measured disturbances in electric and magnetic fields and energetic ions. Because the total gas production rate of comet Halley was expected to be 10 times higher than comet Giacobini–Zinner, direct measurements of comet Halley became plausible (see Fig. 1). This expectation was realized on 22 March 1986 at a distance of $\sim 35 \times 10^6$ km. Thus, ICE, the sixth spacecraft to measure comet Halley (the Giotto encounter occurring on 14 March), both opened and closed the first chapter in the direct exploration of comets.

Measurements of magnetic field fluctuations clearly associated with the comet are shown in Fig. 2. The data are from the plasma wave experiments on ICE (principal investigator F.L. Scarf, TRW, Inc). Evidence for detection of the comet was also obtained by the energetic particle anisotropy spectrometer on ICE (principal investigator R. Hynds, Imperial College, UK). Direct measurement of ion beams channelled to the spacecraft from the comet by the interplanetary magnetic field lines at times later than closest approach is also a possibility (Fig. 1). The exact dates of measurement for comet Halley require data processing which is in progress.

Scarf also reports a possible detection of disturbances from comet Halley by the plasma wave experiment of the Pioneer Venus Orbiter (PVO) around the time of the comet's closest approach to Venus in early February 1986.

Visual wavelength observations

Ground-based images of comet Halley could not be obtained during an interval of some 6 weeks near perihelion when the comet was in angular proximity to the Sun. This gap was filled by the coronagraph/polarimeter (C/P) experiment on the Earth-orbiting Solar Maximum Mission (SMM) (principal investigator R.M. MacQueen, High Altitude Observatory, Boulder, Colorado; the comet observations were obtained under the auspices of an SMM guest investigation headed by

M.B. Niedner). The goal was to obtain a record of the comet's overall structure during the interval when no other visible-light imaging of the comet would be possible.

Because of its design as a solar instrument, the C/P's strong rejection of stray light permitted observations of the comet down to solar elongation of 10° . In principle, a gap of only 10 or 11 days would result when the comet penetrated the limit on 1 February and re-emerged on 11–12 February; Halley's perihelion was on 9 February. The original C/P timeline included comet observations starting on 10 January and ending in early March. These start and finish dates were set by spacecraft constraints established during 1985 engineering tests at a series of large offpoint angles for which SMM was not designed. Several days before the first scheduled observations two of the on-board computer memory cells failed, probably as a result of a cosmic-ray hit. Alternative operational procedures were implemented by the SMM operations team and only two and a half weeks of observations were lost.

Comet Halley observations began on 26 January, and the images taken through

the 'wide-band blue' filter showed an extended coma elongated approximately in the anti-solar direction. The best of the pre-perihelion observations appear to have been taken on 28 January, and in these images there is definite evidence for both a plasma and a dust tail. The angular extent of the plasma tail was not large, around 0.5° , but the tail direction was strongly foreshortened at this time, resulting in a detected linear length of millions of kilometres. Detailed statements on structures and dimensions require full image processing which has not yet been completed.

The post-perihelion observations were degraded by the loss of the SMM high-gain antenna system. As a result of this second operational setback, it was not possible to carry out the comet observations using the tracking and data relay satellite (TDRS) in the normal manner. Reliance was on ground-station contacts. Many of the images show a plasma and a dust tail, similar in quality to the 28 January images; these should prove useful in correlative studies of the solar wind and comets. The last observation of the comet was made on 28 February 1986. Thus, the SMM observations should be useful in

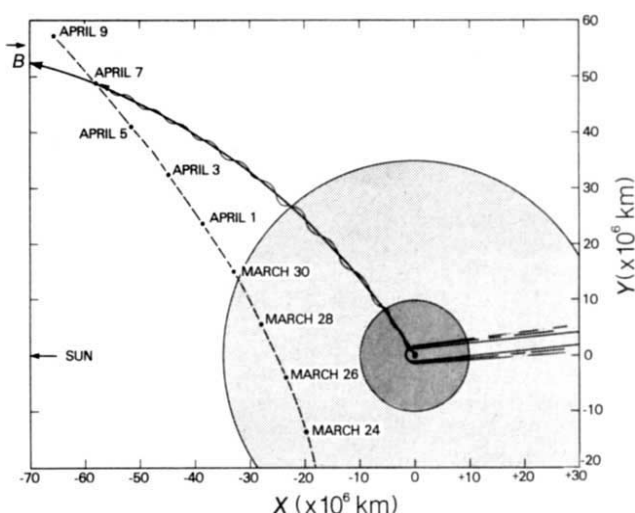


Fig. 1 The trajectory of the ICE spacecraft at closest approach to comet Halley. The distances are plotted in the comet's orbital plane. During the time interval shown, the spacecraft's distance above the orbital plane was $16.6\text{--}5.5 \times 10^6$ km. The inner circle around the comet is 10×10^6 km in radius, representing the hydrogen cloud. The outer circle is 35×10^6 km in radius, representing the total comet/solar-wind interaction region based on measurements from Vega and ICE. The arc between the comet and ICE on 7 April illustrates the possibility of particle beams being channelled to the spacecraft. The connection shown is for a steady solar wind at 475 km s^{-1} .

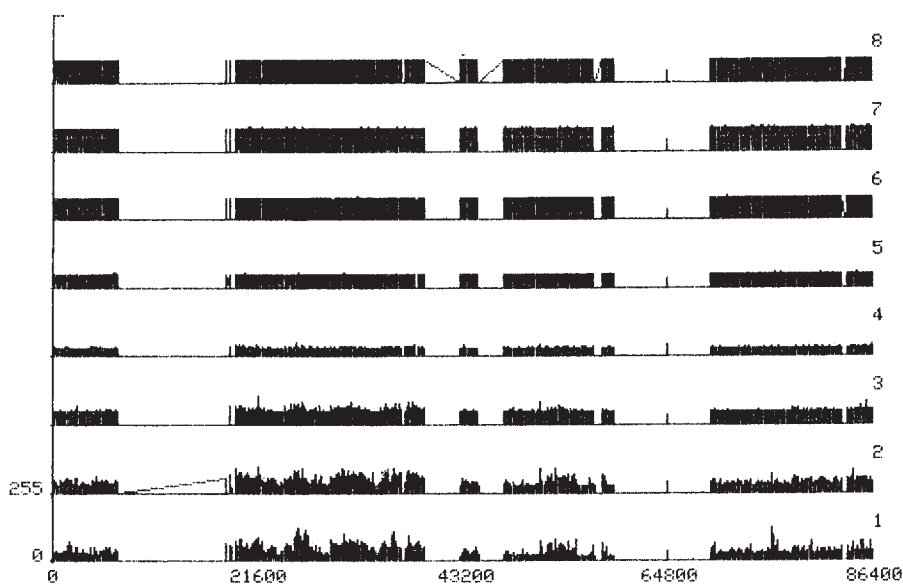


Fig. 2 Detection of enhanced magnetic wave levels on 26 March 1986, indicating traversal of the vast pickup region where cometary ions interact with the solar wind. The frequency sampled varies from 17.8 Hz for channel 1 to 1 kHz for channel 8, and the minimum to full scale spans 80 dB (0 to 255) for each channel. The abscissa is time on 26 March in s. (Courtesy: F.L. Scarf, TRW, Inc.)

providing a record of the structure and evolution of the comet during the last two weeks of February. Ground-based observations will be used to continue the record into March.

Additional visual-wavelength imaging was planned for encounter week from the Astro 1 observatory. The scheduled 6 March launch was cancelled after the loss of Challenger.

Ultraviolet observations

The first IUE star-tracker image of comet Halley was obtained on 11 September 1985, and the ultraviolet spectrum, which showed OH emission, was recorded on 12 September 1985. These are believed to be the first observations of comet Halley from space. The comet was observed every month during the rest of 1985. Due to the comet's angular proximity to the Sun in January and February 1986, it was not observed with IUE. The observations were not scheduled to resume until 13 March when they would support the Giotto encounter. However, when Astro 1, NASA's primary mission to study Halley in the vacuum ultraviolet, was cancelled due to the loss of Challenger, additional IUE observations (requiring new operational techniques) were added. The result was an intensive programme during the week of the Vega and Giotto encounters. Observations are planned to monitor the comet through mid-July 1986; IUE has scheduled 280 hours of observation for comet Halley, more than the telescope has devoted to any previous target in the past year.

Ultraviolet observations provide a means of determining the temporal behaviour of the primary constituents of the gaseous coma. Observations were

made from the Goddard Space Flight Center by both the US team (P.D. Feldman, M.F. A'Hearn, H.A. Weaver and L.A. McFadden) on 9, 11, and 16 March, and by members of the European team (M.C. Festou, C. Arpigny and A.C. Danks) on 12, 13, and 14 March. A sample spectrum obtained on 11 March 1986 is shown in Fig. 3. The IUE fine error sensor, used for target acquisition and tracking, was used in a photometer mode to provide nearly continuous light curves over an 8-hour observing shift, a unique capability. During the encounter week, variations in brightness of a factor of two (in a region $\approx 7,000$ km in radius projected at the comet) were found in a 24-hour period. At the time of the Giotto encounter, this activity was seen to be near a minimum. Preliminary analysis of the ultraviolet spectra, which are qualitatively similar to those of other comets observed by IUE during the past 6 years,

shows similar day-to-day variations in both the gaseous emissions and in the continuum of sunlight reflected by cometary grains. The ultraviolet data can also be used to derive an estimate of the total gas production rate (assuming the gas to be primarily H_2O) even though the models generally used for this purpose assume a steady-state gas production. This is clearly not the case for Halley. At the time of the Giotto encounter, the gas production range was 5×10^{29} molecules s^{-1} , with an uncertainty of about a factor of 2. Additionally, the IUE data provide column abundances of carbon, oxygen, sulphur, OH, CO, CS, C_2 , and CO^+ to compare with the *in situ* measurements of these species by Vega 2 and Giotto.

The hydrogen cloud surrounding comet Halley has been observed by the ultraviolet spectrometer orbiting Venus on PVO (principal investigator, I.A.F. Stewart, University of Colorado). Detailed observations were made for about 5 weeks in February and March 1986. The spectrometer was used as a spin-scan photometer to build up a Lyman- α image of the entire comet. A unique capability of PVO lies in the fact that, when the comet was behind the Sun near perihelion as seen from Earth, the view from Venus was unobstructed.

The data indicate an extensive hydrogen cloud extending to well over 10×10^6 km from the nucleus, as illustrated in Fig. 4. The derived water production rate for the comet showed very large changes around the time of perihelion, in contrast to a relatively quiescent period in late December 1985 and early January 1986. The trend of the measurements indicated that the total water molecule production rate would be $\approx 1 \times 10^{30}$ s^{-1} during encounter week. The last PVO observations of comet Halley were made on 7 March 1986.

The VUV-wavelength imaging photometer on DE 1 (in Earth orbit) has been used to map the comet's hydrogen cloud in Lyman- α emission were observed in January 1986. Extensive observations

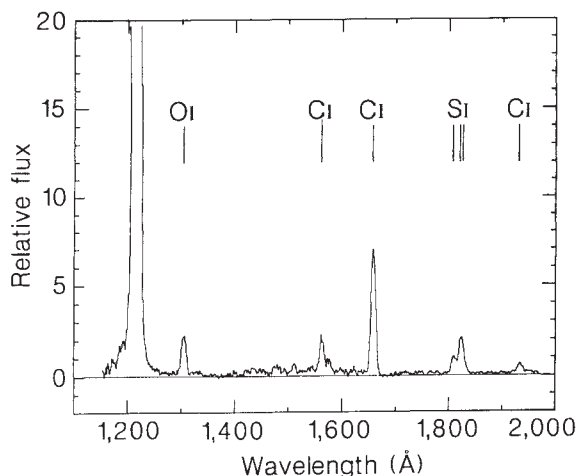


Fig. 3 Preliminary IUE spectrum of comet Halley on 11 March 1986. The saturated feature at 1,216 Å is hydrogen Lyman- β . (Courtesy: P.D. Feldman, Johns Hopkins University.)

were made from 27 February to 20 March 1986 and a last observing period is scheduled for 22 April into June, if possible.

Rocket observations

A sounding rocket experiment specifically designed for ultraviolet spectroscopy of comets and other extended astronomical sources was successfully flown to observe comet Halley from White Sands Missile Range on 26 February at 5:02 MST (12:02 UT). The scientific payload was recovered, refurbished in the field, and reflown on 13 March 1986 at 4:20 MST (11:20 UT), some 13 hours before the encounter of Giotto with comet Halley. The payload, designed and built at the Johns Hopkins University (P.D. Feldman, T.N. Woods, K. Dymond, and D. Sahnou) consists of a 40-cm diameter Dall-Kirkham telescope and Roland circle spectrograph with an ellipsoidal grating. The two-dimensional photon counting detector provides long-slit spectra of the comet with a projected slit length of 5×10^5 km and a resolution of 10^4 km and a spectral range from 1,200 to 1,750-Å, with $10\text{\AA}$ spectral resolution. The primary objective of the experiment was to study carbon chemistry in the gaseous coma by measuring the spatial distribution of CO, C and C^+ , all of which are detected by resonance fluorescence in this spectral region. Furthermore, by using the Earth's limb to occult the Sun, the rocket observation was possible near perihelion when the comet is most active and the effects of solar photodissociation and photoionization (which vary as the inverse square of the distance from the Sun) are most pronounced. Both rocket flights produced excellent quality data clearly showing, in addition to the strong H I (Lyman- β) O I, and C I emissions, several CO fourth posi-

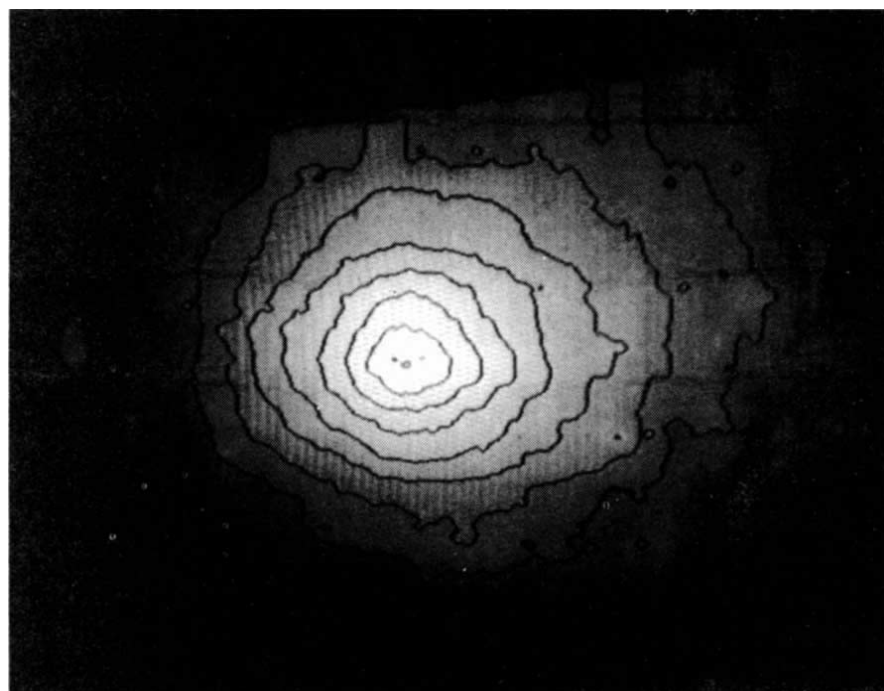


Fig. 4 The hydrogen cloud of comet Halley as observed in Lyman- β by the PVO from 2 to 6 February 1986. The solar direction is to the lower left; the size of the image shown is 0.15×0.15 AU. The crowding of the isophotes on the solar side, and their separation on the anti-solar side, clearly show the effects of solar radiation pressure on the escaping hydrogen atoms; for quiet solar conditions this pressure produced an anti-sunward acceleration. The linear 'edge' near the top is an artefact of the image processing due to a large data gap on 3 February. (Courtesy I.A.F. Stewart, University of Colorado.)

tive bands and the C II emission at 1,335 Å extended into the tail. Also present in the spectra are several weak features, some probably due to atomic carbon, not identified in previous cometary spectra.

An experiment to obtain images and objective spectra of comet Halley was developed by a team consisting of R.P. McCoy, G.R. Carruthers and R.R. Meier (Naval Research Laboratory), C.P. Opal (University of Texas), and M.

Silbert (NASA/Goddard Space Flight Center-Wallops). One electrographic camera obtains images at Lyman- β with a 20° field-of-view and 3 arc min resolution. The second camera points at a grating and obtains objective spectra with a 12° field of view and 1 arc min resolution.

The experiment was successfully flown from White Sands Missile Range on 24 February 1986 at 4:53 MST (11:53 UT) and on 13 March 1986 at 3:54 MST (10:54 UT). Sample results are shown in Fig. 5.

Conclusions

These diverse measurements and observations of comet Halley should greatly contribute to our physical picture of the comet as it appeared during the 1985–86 apparition. Detailed results will be published by the investigators. Additional contributions will come from a comparison with the results obtained by ICE¹ on the tailward side of comet Giacobini–Zinner during the 11 September 1985 flyby.

The excellent cooperation of the investigators mentioned, demonstrated by their rapid communication of results, is gratefully noted. □

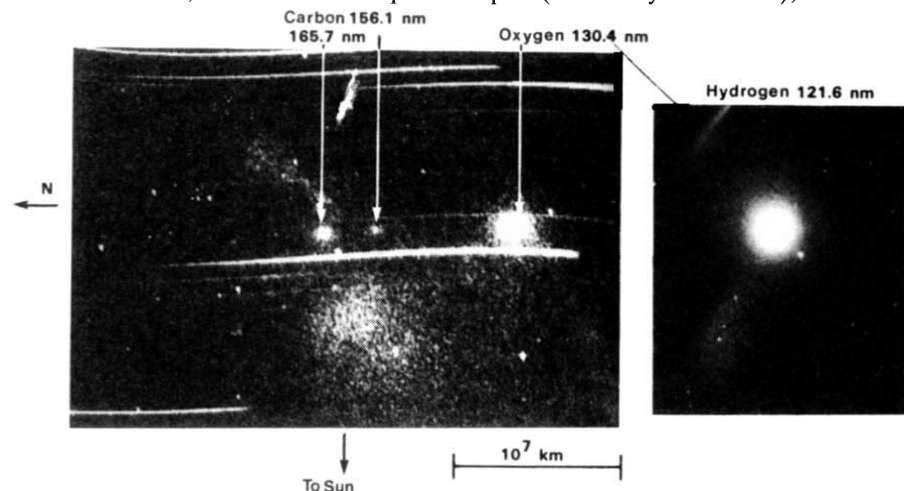


Fig. 5 Far-ultraviolet images of comet Halley obtained by electrographic cameras flown on a rocket launched 24 February, 1986. On the right is a direct image of hydrogen Lyman- β . It shows an extensive coma of hydrogen surrounding the nucleus of the comet and extending out a few million kilometres. On the left, to the same scale, is an objective spectro-gram showing images of the resonance line of atomic oxygen at 130.4 nm and the carbon resonance lines at 156.1 nm and 165.7 nm. The horizontal streaks in the image are the spectra of bright ultraviolet stars. North is to the left, and the Sun is below the images. (Courtesy R.P. McCoy, Naval Research Laboratory).

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Chaos in light

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Chaos is an inherent feature of many nonlinear systems. In particular, the transition from a steady to chaotic state occurs independently of the physical properties of the system. Such behaviour occurs in optics, both in lasers and in nonlinear optical devices. Such devices, which are fundamentally simple both in construction and in the mathematics that describes them, provide excellent opportunities for investigating nonlinear phenomena as well as for technological innovation.

MATHEMATICAL discoveries have revolutionized our understanding of nonlinear science. The preconception that physical systems, in general, behave in a predictable manner is seriously in question. Rather than yielding regular and repeatable behaviour, many nonlinear systems also exhibit unstable, even chaotic, solutions. Furthermore, the transition from stable to chaotic behaviour, which may occur when varying a control parameter of the system, follows specific, well-defined routes which are universal in the sense that they are independent of the physical properties of the system they describe. It is these signatures which have been a major impetus to experimentalists in the subsequent search for physical systems that exhibit these phenomena. Such behaviour has now been observed in many branches of science and the number is rapidly growing.

The most recent exciting development is the discovery that such phenomena exist in optics. Here we are concerned, in general, with the nonlinear interaction of light with media contained in optical resonators; the physical properties of the medium, such as absorption and refractive index, being modified by the intensity of the incident radiation.

There are two major areas. The first are lasers or active systems, in which the optical signal is derived from stimulated emission generated within an optical cavity containing a gain medium. The second are passive systems for which the optical signal is but the transmission of an input light signal through an optical cavity containing, for example, an absorptive medium.

Instabilities in laser emission, notably in the form of spontaneously coherent pulsations, have been observed almost since the first demonstration of laser action. However, subsequent theoretical efforts towards understanding these phenomena have been at a modest level, due in part to the wide variety of alternative areas of investigation provided by lasers. It is only with the new mathematical discoveries that these instability phenomena have been investigated to give a deeper insight into the mechanisms of laser action and its deterministic chaotic behaviour. Re-examination of many of these systems show that such effects are quite abundant; the operating window for conventional stable emission in some systems often proving surprisingly small while in some cases, the instabilities are found to prevail just where the lasing emission is optimum.

On the other hand, passive systems which are being increasingly recognized for their potential application as bistable all-optical logic elements, may give rise to similar phenomena. It may, nevertheless, be possible to take advantage of the periodic instabilities that precede chaos in the development of ultra-high frequency all-optical modulators.

Of the variety of physical systems that exhibit deterministic instability phenomena, optical systems, both lasers and passive devices, provide nearly ideal systems for quantitative investiga-

tion due to their simplicity both in construction and in the mathematics that describe them, enriched by the possibility of a quantum description. Notable also is the very short timescale (nanosecond to microsecond) over which optical instabilities occur which, in contrast to many other systems, ensures essentially constant environmental conditions during data acquisition. This is particularly important since even small extraneous perturbations, such as noise, may dramatically alter the form of the subsequent temporal evolution of the instability process. These features are fundamental to the rapid establishment of optical systems in this multidisciplinary field.

Universality in chaos

In considering deterministic behaviour, one is tempted into the misconception that such behaviour must be regular since successive states evolve continuously from each other. However, as early as 1892 Poincaré showed that particular mechanical systems, where time evolution is governed by hamiltonian equations, could display chaotic behaviour. The subsequent discovery by Lorentz¹ in 1963 that even a simple set of three coupled first-order, nonlinear differential equations can lead to completely chaotic trajectories is recognized as a landmark. This work is fundamental to our understanding of laser instabilities.

At first sight, such behaviour appears alien to our conception of many problems in physical science. This prejudice stems from the dominance of mathematical theory pertaining to linear systems and its subsequent successful application to many fundamental linear problems in the physical sciences. Unfortunately, however, this has led to a narrow vision of the physical world where nonlinear behaviour is the rule and linear behaviour the exception.

Unlike linear systems, nonlinear systems must be treated in their full complexity, and so there is no general analytical approach for solving them. The advances made in understanding many previously intractable nonlinear problems can largely be attributed to the power of contemporary computers, where simulated solutions of nonlinear equations have provided insights into their behaviour and suggested directions for future research.

The temporal evolution in the behaviour of a system can be characterized when presented as a trajectory of a point in the phase space of its dynamical variables. In this representation, consider a familiar dynamical system such as a periodically-forced pendulum in a frictional environment. Such a dynamical system is characterized by the fact that the rate of change of its variable is given as a function of the value of the variable at that time. The space defined by the variables is called the phase space. The pendulum's behaviour can be described by the motion of a point in a two-dimensional phase space whose coordinates are the position and velocity of the pendulum. In

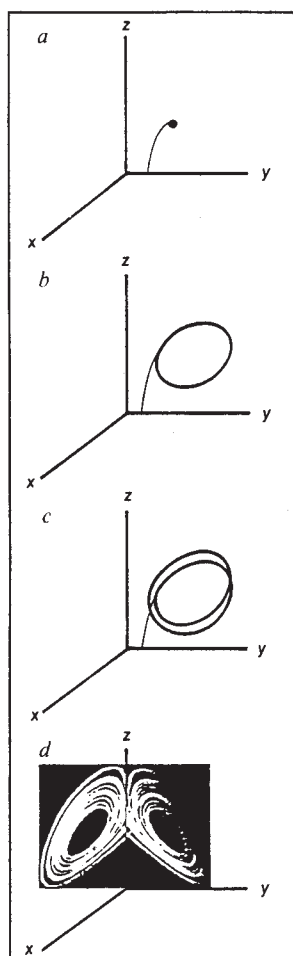


Fig. 1 Phase space portraits of the attractor for the dynamical variables x , y and z . *a*, Stable point corresponding to a steady state in time after initial transients have died out shown as the thin continuous line. *b*, Period-one limit cycle corresponding to a periodic solution in time of single frequency. *c*, Bifurcation to period-two limit cycle; a periodic solution in time with double the period of that in *b*. Successive period doubling bifurcations lead to an eventual chaotic solution. *d* Shows the strange or chaotic attractor for a Lorenz-Haken system describing a single mode laser with a homogeneously broadened two-level gain medium.

more complicated systems, involving many variables, the dimension of the phase space will, however, be considerably larger. If an initial condition of a dissipative dynamical system, such as the pendulum, is allowed to evolve for a long time, the system, after all the transients have died out, will eventually approach a restricted region of the phase space called an attractor. A dynamical system can have more than one attractor in which case different initial conditions lead to different types of long-time behaviour.

The simplest attractor in phase space is a fixed point. The system is attracted towards this point and stays there. This is the case for a simple pendulum in the presence of friction; regardless of its initial position, the pendulum will eventually come to rest in a vertical position. When the pendulum is under the influence of an external periodic driving force, the system is then fully nonlinear and leads to strikingly different behaviour. Irrespective of the initial conditions the pendulum always ends up making a periodic motion. The limit or attractor of the motion is a periodic cycle called a limit cycle. However, when the driving force exceeds a certain critical value, the periodic motion of the pendulum breaks down into a more complex chaotic pattern

which never repeats itself. This motion represents a third kind of attractor in phase space called a chaotic or strange attractor (see Fig. 1).

A trajectory on a chaotic attractor exhibits most of the properties intuitively associated with random functions, although no randomness is ever explicitly added. The equations of motion are purely deterministic; the random behaviour emerges spontaneously from the nonlinear system. Over short times, the trajectory of each point can be followed, but over longer periods small differences in position are greatly amplified making the predictions of long-term behaviour impossible. As such arbitrarily close initial conditions can lead to trajectories which after a sufficiently long time diverge widely; even for the simplest of systems in which all the parameters are determined exactly, long-term prediction is, therefore, impossible. This behaviour is in marked contrast to that of the fixed point and limits cycle attractors for which, irrespective of starting conditions, the system always settles down to the same solutions.

Irratic and aperiodic temporal behaviour of any of the systems' variables implies a corresponding continuous spectrum for its Fourier transform which is, therefore, also a further signature of chaotic motion. However, other factors including noise, can lead to continuous spectra, and distinguishing chaos from noise is one of the major problems of the field. Hence, although time series, power spectra and routes to chaos collectively provide strong evidence of deterministic behaviour further signatures are desirable for its full characterization and in discriminating it from stochastic behaviour. Here analysis of trajectories of a point in the phase space of its dynamical variables is required. However, for a system with, say, N degrees of freedom it seemed that it would be necessary to measure N independent variables; an awesome if not impossible task for complex system. Consequently mathematicians have long tried to develop practical techniques for extracting specific finite dimensional information from the limited output provided by experiment; typically the time record of a specific physical observable; that is, one variable of the system. Here embedding theorems² have been recently used to reconstruct phase portraits from which Lyapunov exponents may be determined that measure the average rate of exponential separation or contraction of nearby points on the attractor. These measure intrinsically dynamical properties, unlike power spectra, and provide quantitative measures by which chaotic motion may be distinguished from stochastic behaviour.

The discoveries that deterministic chaos proceeds through a limited number of specific routes when a control parameter of the nonlinear system is varied is profoundly significant as such behaviour is not restricted to a particular model description of a particular physical system. Rather, nonlinear physical systems in all branches of science which may be formally described by the same set of mathematical equations will give solutions that evolve identically in time through one or other routes to chaotic motion. The unique effect of such unification between many separate scientific disciplines forms the basis for the foundation of synergetics^{3,4}. There are at least three common routes by which a nonlinear system may become chaotic. These are referred to as period doubling, intermittency and two-frequency scenarios.

Period doubling. From considering various difference equations, many of which can be reduced to simple one-dimensional maps, solutions have been found to oscillate between stable values, the period of which successively doubles at distinct values of the external control parameter^{5,6,7}. This continues until the number of fixed points becomes infinite at a finite parameter value, where the variation in time of the solutions becomes irregular (see Fig. 1). One example showing such behaviour is the simple logistic map

$$X_{n+1} = rX_n(1 - X_n)$$

Perhaps the most popular application of this map is in describing

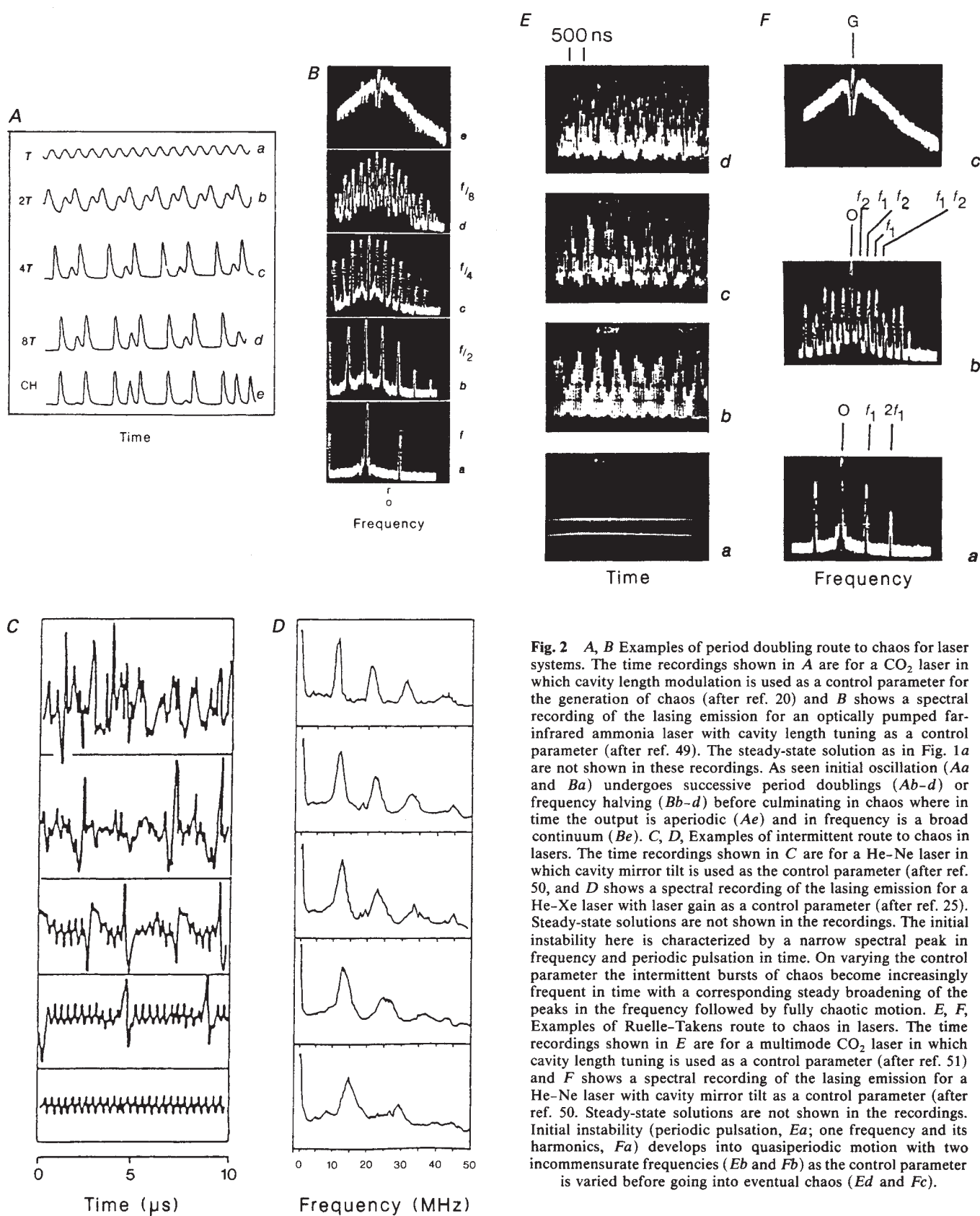


Fig. 2 *A, B* Examples of period doubling route to chaos for laser systems. The time recordings shown in *A* are for a CO₂ laser in which cavity length modulation is used as a control parameter for the generation of chaos (after ref. 20) and *B* shows a spectral recording of the lasing emission for an optically pumped far-infrared ammonia laser with cavity length tuning as a control parameter (after ref. 49). The steady-state solution as in Fig. 1a are not shown in these recordings. As seen initial oscillation (*Aa* and *Ba*) undergoes successive period doublings (*Ab-d*) or frequency halving (*Bb-d*) before culminating in chaos where in time the output is aperiodic (*Ae*) and in frequency is a broad continuum (*Be*). *C, D*, Examples of intermittent route to chaos in lasers. The time recordings shown in *C* are for a He-Ne laser in which cavity mirror tilt is used as the control parameter (after ref. 50), and *D* shows a spectral recording of the lasing emission for a He-Xe laser with laser gain as a control parameter (after ref. 25). Steady-state solutions are not shown in the recordings. The initial instability here is characterized by a narrow spectral peak in frequency and periodic pulsation in time. On varying the control parameter the intermittent bursts of chaos become increasingly frequent in time with a corresponding steady broadening of the peaks in the frequency followed by fully chaotic motion. *E, F*, Examples of Ruelle-Takens route to chaos in lasers. The time recordings shown in *E* are for a multimode CO₂ laser in which cavity length tuning is used as a control parameter (after ref. 51) and *F* shows a spectral recording of the lasing emission for a He-Ne laser with cavity mirror tilt as a control parameter (after ref. 50). Steady-state solutions are not shown in the recordings. Initial instability (periodic pulsation, *Ea*; one frequency and its harmonics, *Fa*) develops into quasiperiodic motion with two incommensurate frequencies (*Eb* and *Fb*) as the control parameter is varied before going into eventual chaos (*Ed* and *Fc*).

changes in population ($X_n \rightarrow X_{n+1}$) of an organism or species from year to year ($n \rightarrow n+1$) where there is no overlap between successive generations. The first term in the brackets describes population growth by birth and the second term, population decline due, for example, to predatorial or other environmental conditions. Dependent on the increase in the value of the control parameter r the population may be steady, oscillatory or chaotic the transition following a period doubling scenario (see refs 8, 9). More generally many complex physical systems, often described by large numbers of coupled differential equations, may be reduced in some conditions to the form of this or similar maps. Period doubling bifurcation to chaos has been experimentally observed in numerous systems (see Fig. 2A, B).

Intermittency. Intermittency¹⁰ means that a signal which behaves regularly in time becomes interrupted by statistically-distributed periods of irregular motion. The average number of these intermittent bursts increases with the external control parameter until the condition becomes completely chaotic (see Fig. 2C, D).

Two frequency. Turbulence in time was originally considered as a limit of an infinite sequence of instabilities (Hopf bifurcation) evolving from an initial stable solution each of which creates a new basic frequencies^{11,12}. However, it has been recently shown^{13,14} that after only two or perhaps three instabilities in the third step the trajectory becomes attracted to a bounded region of phase space in which initially closed trajectories separate exponentially; as such the motion becomes chaotic (see 2E, F).

Chaos in lasers

Chaotic behaviour in lasers may exist in even the simplest of systems: one in which population inversion is established between two discrete energy levels of the medium and where the lasing transition between these two levels is homogeneously broadened. A variety of practical lasers may be controlled to operate in these conditions. A further simplification is that the laser cavity, a Fabry-Perot or ring resonator system surrounding the gain medium, be sufficiently short so that only one resonant frequency of the cavity lies within the bandwidth of the gain medium and that this mode be resonantly tuned to the gain centre frequency. The frequency spacing ($\Delta\nu$) between cavity modes for a Fabry-Perot cavity is given by:

$$\Delta\nu = cn/2L$$

where c is velocity of light, n the refractive index of the lasing medium and L the cavity length. Typical examples of single mode and many mode operation, for short and long cavity lengths respectively, are shown in Fig. 3. In conditions in which the gain or population inversion is maintained at a constant level by, for example, constant electrical or optical excitation, and for the single mode system, lasing occurs with a constant output power at the frequency of the single cavity mode. In this condition, the gain is reduced to a threshold level equal to the cavity losses. Note that even for a multimode system and in the absence of spatial hole burning, lasing is still restricted to a single mode, because the mode with highest gain, here at or near gain centre, grows at the expense of all other modes. Such behaviour is the accepted operating characteristics of these systems.

However, the discovery¹⁵ that for certain operating conditions emission could be periodic or even chaotic implies that the signal comprises more than one frequency, contrary to the accepted understanding of single mode operation. Prediction of such behaviour were initially identified by Haken through the mathematical equivalence of the equations describing laser action, the Maxwell-Bloch equations, and those derived earlier by Lorenz to describe chaotic motion in fluids. If we consider the trajectory of the Lorenz strange attractor (see Fig. 1d) where in the equivalent laser system the dynamic variables x , y and z are the field amplitude (E), polarization of the medium (P),

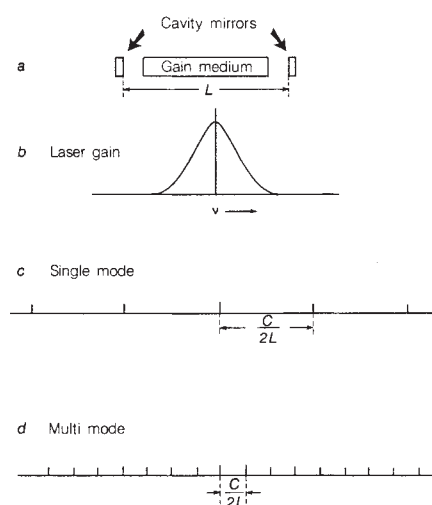


Fig. 3 a, A Fabry-Perot laser cavity system with a partially transmitting mirror for coupling out the laser emission; b, Lorentzian gain profile for a simple two-level homogeneously broadened lasing medium for which each and every atom/molecule emits identically. c, Relative position of the cavity modes for a short optical cavity. Here only one mode lies within the gain bandwidth resulting in a single mode emission, d, Corresponding position of the modes for a long cavity for which several modes lie within the gain bandwidth.

and the population inversion (D), a point (x, y, z) circles in one region for a while, but then suddenly jumps into another region, where it moves for a while until it jumps, seemingly randomly, back into the first region, and so on; the trajectory never intersects. For the laser, such behaviour not only requires a cavity with high transmission but also a gain of at least nine times that required to produce lasing, making the experimental realization of such operation rather impracticable for most lasers of this simple type. A notable exception are optically-pumped far-infrared molecular lasers¹⁶ which will probably become an important research area.

General prerequisites for the onset of deterministic chaos include: that apart from nonlinear interaction there is a sufficiently large phase space; the minimum requirement being that the system possesses at least three degrees of freedom. For example, the simple pendulum possesses only two degrees of freedom and exhibits at most periodic behaviour. In a two-dimensional phase space, described by the variables, velocity and displacement, it is then impossible for the trajectory to be restrained within a basin of attraction without intersecting itself; repetition of trajectory points resulting in only periodic or quasiperiodic solutions. The addition of one further degree of freedom, through the imposition of a modulated external force, leads to the possibility of a non-overlapping trajectory still in a limited phase space but now described by three variables; the so-called strange attractor (see ref. 17).

The Maxwell-Bloch equations described above for the special case of a single-mode laser with field tuned to the centre of the gain line such that both field and polarization are real quantities, satisfy the minimum condition of three independent variable equations, each of which has its own relaxation.

$$\frac{dE}{dt} = -\kappa E + \kappa P$$

$$\frac{dP}{dt} = \gamma_{\perp} E D - \gamma_{\perp} P$$

$$\frac{dD}{dt} = \gamma_{\parallel}(\lambda + 1) - \gamma_{\parallel} D - \gamma_{\parallel} \lambda E P$$

where κ is the cavity decay rate, $\gamma_{\perp}$ is the decay rate of atomic

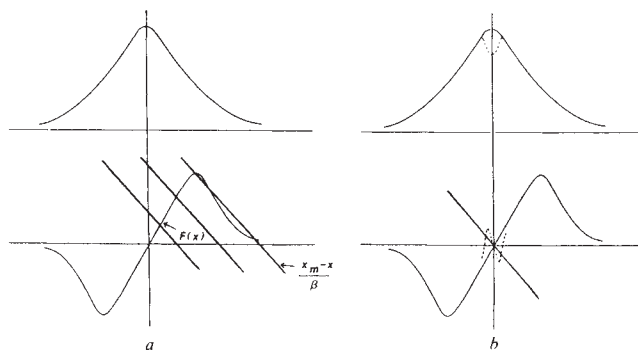


Fig. 4 Graphical solution of the equation of active cavity mode: $F(x) = (x - x_m)/\beta$. Top traces represent gain profile and the corresponding dispersion features associated with these is shown in the bottom traces. *a*, Graphical solution is illustrated for different detunings of the cavity mode. As seen only when the mode is tuned sufficiently away from the line centre, intersection on more than one point is possible resulting in the splitting of the mode into different frequencies all of which fill the same number of half-wavelengths between the cavity mirrors; spontaneous or passive mode splitting. *b*, When the oscillating cavity mode burns a spectral hole into the gain, the associated dispersion takes just the shape required for giving rise to more than one intersection point. As seen, the gain at the split frequencies are indeed higher than that at the original frequency; induced or active mode splitting.

polarization, $\gamma_{\parallel}$ is the decay rate of population inversion, λ is the pumping parameter, E is the field inside the cavity, D is the population inversion, and P is the atomic polarization.

However, if one variable relaxes faster than the others the stationary solution for that variable may be taken, so resulting in a reduced number of coupled differential equations; commonly termed adiabatic elimination of the fast variables^{3,4}. In many systems, polarization and population inversion have relaxation times much shorter than the cavity lifetime and both variables can be adiabatically eliminated. With just one variable describing the dynamics, the laser must show a stable behaviour (fixed point in phase space; see Fig. 1*a*). This group of lasers, comprises many common systems such as He-Ne, Ar⁺, Dye and lasers. In some cases, only polarization is fast and hence two variables describe the dynamics. In this class, we find ruby, Nd and CO₂ lasers which exhibit oscillating behaviour in some conditions, although ringing is always damped.

Since many lasers are not described by the full set of Maxwell-Bloch equations normally chaotic behaviour from these systems cannot be obtained. For these systems with less than three variables the addition of independent external control parameters to the system, as for the pendulum considered above, have been extensively considered¹⁸ as a means to provide the extra degrees of freedom. Active modulation of a parameter such as population inversion, field, or cavity length as well as injection of a constant field detuned from the cavity resonance and also the use of intracavity saturable absorbers have all been considered¹⁹⁻²¹. For multimode rather than single-mode lasers intrinsic modulation of inversion (or photon flux) by multimode parametric interaction ensures additional degrees of freedom¹⁸. When the field is detuned from gain centre the field amplitude, polarization and population inversion are complex, providing (in the absence of adiabatic elimination) five rather than three nonlinear equations for single mode systems which is more than sufficient to yield deterministic chaos for suitable parameter values²². Also of significance is the remarkably low threshold found for the generation of instabilities and chaos in single mode inhomogeneously broadened laser systems²³⁻²⁵. Compared with homogeneously-broadened systems this is attributed to the increased number of independent gain packets available in inhomogeneous systems. Pulsating instabilities and routes to

chaos have also been reported for Raman lasers²⁶ where the instability threshold is again found to be reduced. Significantly, it is also found that instabilities are greatest in conditions for which the laser produces maximum output.

Physical mechanism

A transition from a steady laser output to an oscillatory and subsequently chaotic emission implies the generation of further oscillating frequencies to that of the original stable emission. That this should occur even for a so-called single frequency (single mode) laser seems to be a contradiction in terms. The explanation is in the phenomena of mode splitting^{27,28}. This occurs in a region of rapidly varying dispersion when the oscillating cavity mode splits into more than one frequency all of which fill the same number of half-wavelengths between the cavity mirrors. Coupling between these several frequencies having common mode index is a prerequisite for single-mode pulsating instabilities. Dispersion changes its value rapidly near the wings of the gain curve and the splitting that occurs in this region is known as passive mode splitting. An oscillating cavity mode can also induce large variation in dispersion if it can locally saturate the gain curve, hence this splitting is termed induced mode splitting.

To understand spontaneous mode splitting we consider the equation of the oscillating active cavity mode

$$\frac{mc}{2L} = \nu n(\nu) \quad (1)$$

where, m is the mode index and $n(\nu)$ is the index of refraction at the laser oscillation frequency ν . In terms of the empty resonator mode frequencies, $\nu_m = mc/2L$ obtained by putting $n(\nu) = 1$ the above equation may be re-written as,

$$\nu_m - \nu = \nu[n(\nu) - 1] \quad (2)$$

When the value of $n(\nu)$, either for a Lorentzian or Gaussian gain profile which are common to most lasers, is substituted this equation may be re-expressed as,

$$x_m - x = \beta F(x) \quad (3)$$

where x and x_m are respectively the laser oscillation frequency and empty cavity resonant frequency normalized as a detuning from the atomic resonance. $F(x)$ has the same functional dependence with x as $n(\nu)$ has with ν and it depends on whether the gain is Gaussian or Lorentzian. The mode splitting factor, β , is dimensionless and is given by

$$\beta = K \frac{cg}{(\Delta\nu)} \quad (4)$$

where g is the peak small-signal incremental gain, $\Delta\nu$ is the FWHM (full width at half maximum) value of gain-width, c is the velocity of light in vacuum and K is a numerical factor equal to $\pi^{-3/2}/\ln 2$ for Gaussian gain profile and π^{-1} for Lorentzian gain profile. Equation (3) has been graphically solved for different detunings for a Gaussian gain profile in Fig. 4*a*. Under certain detuning (near the wings of the gain) the equation is simultaneously satisfied by more than one value of x . Physically this means that the cavity mode is split into more than one frequency and all of which correspond to the same number of half-wavelengths within the resonator cavity. Such effects occur spontaneously at the wings of the gain curve and hence the name spontaneous mode splitting.

Induced mode splitting may be explained in terms of distortions in the dispersion caused by hole burning in the gain profile by a single mode operating above lasing threshold (see Fig. 4*b*). The dispersion may be so distorted that several new frequencies satisfy the boundary conditions. The onset of these sideband frequencies (the gain at which can be more than that at the parent oscillating frequency; see Fig. 4*b*) gives rise to pulsations

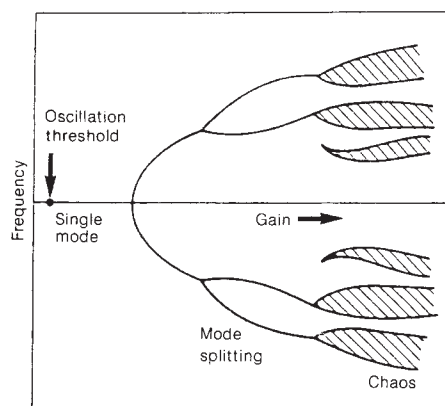


Fig. 5 Mode splitting sequence in which an initially single mode (single frequency lasing oscillation) successively bifurcates on varying gain to generate emission with increasing complex frequency context culminating in a broad band (chaotic) spectrum (after ref. 52).

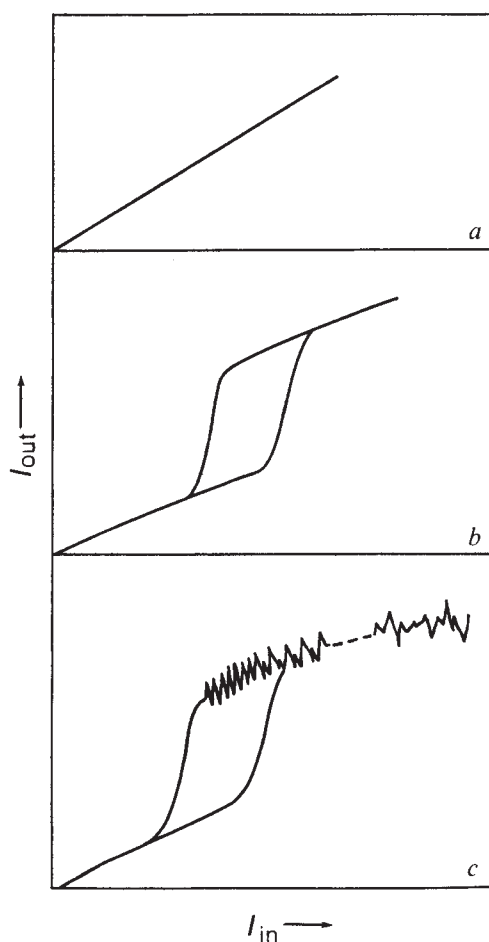


Fig. 6 Transmitted intensity as a function of input intensity for a simple optical resonator, for example, Fabry-Perot or ring cavity. *a*, An empty cavity showing characteristic linear behaviour; *b*, a characteristic optical bistable action and associated hysteresis; *c*, appearance of periodic and chaotic behaviour in the transmitted signal, on increasing the input signal intensity, that may occur in the form of trace *b* for different parameter conditions of the nonlinear cavity.

in the intensity output of the laser. Because the dispersive effects are caused by the oscillating mode itself, this splitting is termed 'induced mode splitting'. This effect should be strongest at line centre and probably exists over much of the lasing tuning range. In view of these effects we may now consider two line broadening situations common to most lasers—homogeneous and inhomogeneous broadening.

The saturating characteristics of an inhomogeneously broadened gain medium is ideal for the realization of mode splitting. In such a medium, unlike the homogeneously broadened case more than one frequency may naturally oscillate since independent sets of atoms/molecules are responsible for lasing at different frequencies across the gain-bandwidth and thus passive mode splitting may be readily observed. The oscillating frequency may also burn a spectral hole into the gain medium thus favouring induced mode splitting. Furthermore, the side bands split again as they again burn spectral holes. This process will continue and may eventually lead to chaos (see Fig. 5). On the other hand, when a homogeneously-broadened gain medium saturates, all the atoms or molecules actually contribute at the oscillating frequency thereby making the possibility of spectral hole burning almost impossible. In such a system, therefore, only the frequency with highest gain would eventually grow. However, for the Haken-Lorentz system, in an extremely high-gain medium survival of more than one frequency is possible.

Chaos in nonlinear optical devices

In parallel with work on laser instabilities, Ikeda predicted oscillation and chaos in passive optical systems²⁹. In these systems, the cavity contains a medium whose refractive index is modified by the intensity of the input light signal. This may be expressed as

$$n(I) = n_0 + n_2 I \quad (5)$$

where n_0 and n_2 are respectively the ordinary and nonlinear contribution towards the refractive index and I is the light intensity.

To understand this phenomena we must consider how the light field within the cavity changes, according to the intensity dependent nonlinear phase shift produced by this refractive index, with successive round trips. The simplest case is one in which the intensity-dependent refractive index immediately responds to changes in intensity and where the cavity is of low finesse. The intensity of the signal inside the cavity, here a ring resonator, for successive round trips then obey the one-dimensional mapping rule³⁰

$$I_{n+1} = I_{in} \left\{ 1 + C \cos \left[\frac{2\pi}{\lambda} (n_2 I_n) L + \phi_0 \right] \right\} \\ = F(I_{in}, C, I_n) \quad (6)$$

where $\phi_0 = (2\pi/\lambda)n_0 L$, is the normal round trip optical phase shift experienced by the input signal in the cavity in the absence of nonlinearity, C is a constant for the particular cavity and we assume that the nonlinear medium fills the whole cavity of length L . The stationary solution of this mapping, denoted by I_s , occurs when $I_{n+1} = I_n$ and yields the relation

$$I_s = I_{in} \left\{ 1 + C \cos \left[\frac{2\pi L}{\lambda} (n_2 I_s) + \phi_0 \right] \right\} \quad (7)$$

where the transmitted signal (I_T) is then simply given by $I_T = I_s T$, where T is the transmission of one of the cavity mirrors.

In the limit of low intensity, the nonlinear term is negligible and the transmitted signal of the ring cavity reduces to the standard expression

$$I_T = I_{in} \{ 1 + C \cos \phi_0 \} T \quad (8)$$

for this optical cavity. Here the intensity of transmitted signal scales linearly with that of the input signal (see Fig. 6*a*) its

magnitude depending on the degree to which the input signal frequency is resonant with the cavity. Tuning the length of the cavity, thereby varying the phase ϕ_0 , leads to the familiar resonant transmission peaks characteristic of optical cavities. Returning to the nonlinear equation a corresponding plot of transmitted intensity as a function of input intensity is shown in Fig. 6b. The nonlinear form of this curve is the signature of optical bistability, of interest because of its application to all optical logic elements. For example, this particular curve shows switching action and memory in which at a critical input intensity the output signal switches to a higher value but on the return cycle switches down for a reduced input intensity due to optical hysteresis. A stable and reproducible operation is required for such application. However, there are parameter conditions, for the input intensity, and cavity finesse for these systems for which the transmitted signal exhibits a behaviour far from this ideal, manifesting not only pulsating instabilities but also full chaotic characteristics. Such regions are schematically indicated on the bistability curve (Fig. 6c).

To understand this behaviour we need to examine more closely the mapping behaviour of equation (6) as shown in Fig. 7a. Intersection of this curve with the line $I_{n+1} = I_n$ gives the stationary solution to the mapping. Starting from an arbitrary value of I_n , the corresponding value of I_{n+1} (vertical intersection with $F(I_n; C; I_n)$) is then the new value of I_n (horizontal intersection with line $I_{n+1} = I_n$). Successive iteration generates a spiral of successively decreasing steps which converge to a single point intersection shown. This is then a stable single point solution. In time, the iteration steps are then transients which die out to this steady-state solution. Similar constructions are shown in Fig. 7b and c for two higher values of C ; the input intensity could, of course, be used as an alternative control parameter. As seen in equation (6) this is manifested as an increase in amplitude of the map. In Fig. 7b, successive iterations, do not converge to a single point but stabilize to a two-point solution; shown by the intersection of the iterations with the mapping function $F(I_n; C; I_n)$. The corresponding signal in time, therefore, shows that after transients have died out the transmitted signal exhibits periodic oscillation or limit cycle behaviour. For further increase in C , the oscillatory signal successively bifurcates to yield oscillations which are each double the period of the preceding ones; the so-called period doubling sequence, as shown in Fig. 2 for laser systems. Ultimately for sufficiently high C a chaotic solution is obtained for which, as seen in Fig. 7d, successive bifurcations never converge to fixed points. This figure also shows that a small change in the starting conditions, value of I_n , will soon result in wildly different iterative steps; unlike the stable and periodic solutions such behaviour which characterizes deterministic chaos is often referred to as being sensitive to initial conditions. As such a small difference in the initial condition is amplified by many operations. Mathematically, this is quantified by the Lyapunov exponent λ which for this system is

$$\lambda = \log \frac{I_n C}{2} \quad (9)$$

In general, a positive Lyapunov exponent implies exponential separation of nearby trajectories, the signature of deterministic chaos, while a negative exponent implies exponential contraction, the signature of an attracting fixed point. For chaotic behaviour one, therefore, requires $I_n C/2 > 1$, whereas bistable action without the presence of instability requires $I_n C/2 < 1$. Evidently a transition to chaos may then occur on increasing the input intensity or the value of the parameter C . For convenience, we have assumed that the medium responds instantaneously to the field or more precisely the relaxation time of the medium is much shorter than the cavity round trip time of the field. For systems in which the converse situation applies bistable operation is favoured although even here sideband instabilities may arise.

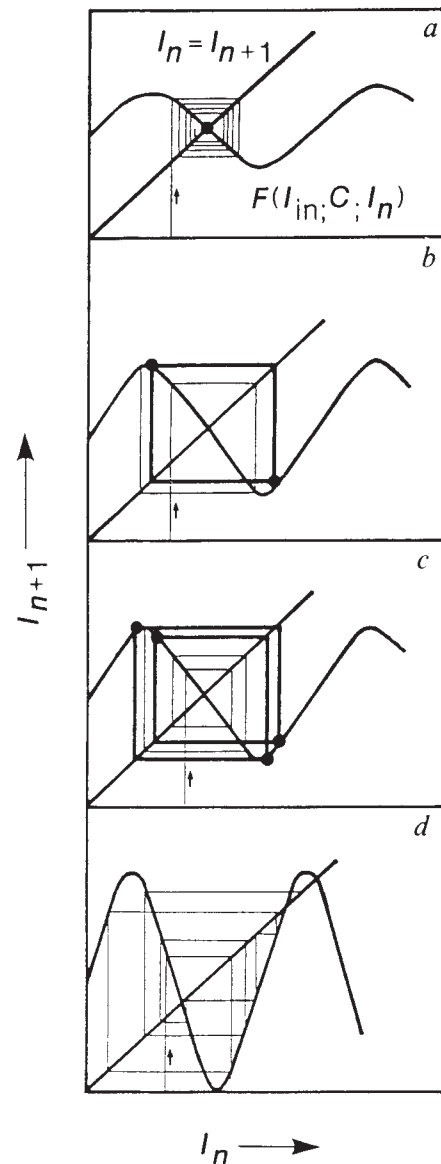


Fig. 7 Graphical solutions of one dimensional map $I_{n+1} = F(I_n, C, I_n)$ for various values of control parameter C . The line $I_n = I_{n+1}$ is the condition when the transmitted signal does not change with successive iterations. a, A fixed point or steady-state solution I_s for the field in the cavity since successive iterations converge to the single intersection point of the two curves. b, An increased value of C , shows the iterations to stabilize on a two-point solution corresponding to a periodic output in time. For further increase in the value of C , a four-point solution is obtained (c); that is iterations here have to cycle twice before repeating themselves so corresponding to a periodic oscillation of twice that for the trace b. This period doubling behaviour repeats as the control parameter C is further increased until a value is reached when iterations no longer repeat themselves. This is shown in the trace d which characterizes an example of chaotic behaviour. We note that the stable and periodic solutions (traces a-c) are invariant with the starting value of I_n . In contrast, the chaotic trajectory of trace d is very sensitive to this initial condition; following a very different path for even the smallest change. The time series of Fig. 2A are representative of the behaviour described here.

Observations of these phenomena, although still limited, have been made in various optical systems such as hybrid bistable devices, and all optical bistable systems. In the hybrid system, nonlinearity in a transparent dielectric medium is caused by an externally-applied voltage rather than by the laser signal itself. The impressed voltage is coupled to the transmitted light signal

through a controllable electronic delay with variable gain which serves as the control parameter of the system. On increasing the feedback gain the transmitted optical signal undergoes a period doubling bifurcation though only up to period eight before becoming chaotic. Here³¹ as in many other systems, both passive and active, the effect of external noise³² interrupts the otherwise infinite series of period doublings to create what is termed a bifurcation gap.

The first observation of bifurcation to periodic and chaotic states in an all optical bistable system was made with a single mode optical fibre as the transparent nonlinear medium in a ring cavity³³, the refractive index here being directly modified by the input intensity through the Kerr effect. Here, the high intensities required to induce sufficient nonlinearity precluded the use of a continuous wave (cw) input source and instead a mode-locked train of intense short pulses, separated in time by an amount equal to the round trip time of the ring cavity, was used. Each pulse is, in effect, an iterative step in which the signal of one pulse in circulating the ring cavity adds to the signal of the next incoming pulse, the process repeating for the whole train. Since bifurcations are caused by the interference between the incident field and cavity field which, suffers a nonlinear phase shift in each round trip of the cavity (see equation (6)), the transmitted signal is evidently modified by the intensity of the pulses. With this system period doubling followed by chaos was observed.

The most fundamental of all nonlinear optical systems is nevertheless, one containing a two-level medium as originally analysed by Ikeda for a ring cavity³³. Near resonant excitation of the medium will, for a sufficiently intense incident signal, cause saturation of the absorption which thereby modifies the associated anomalous dispersion feature of the two-level transition. The mechanism is, in many respects, equivalent to that which gives rise to mode splitting in lasers described earlier for a two-level gain rather than absorptive medium. As for the laser, the intensity dependent change in refraction (dispersion) is resonantly enhanced resulting in considerably reduced power requirements to those used in the fibre experiments. Both atomic and molecular gases are potentially useful media for investigation here, providing discrete levels many of which are in near resonance with available laser lines and approximate reasonably closely to two-level systems. The first corroboration of period doubling routes to chaos in such systems were made in both ring and Fabry-Perot cavities containing ammonia gas near

resonantly-excited by pulsed CO₂ radiation^{34,35}. Recently, experiments have been extended to cw pump laser conditions³⁶ in which sodium vapour was used as the nonlinear medium. Preliminary results show evidence of period doubling with additional instabilities yet to be fully characterized.

Recognizing the problems that chaotic behaviour may pose for fast all-optical bistable elements, yet, operated in the period doubling window and, in particular, period two oscillation, for which the operational parameter window is largest, they offer the attractive prospect of high-frequency all-optical modulators. But such an operation requires that the relaxation time of the nonlinear medium be much faster than the cavity round trip time, then for small devices (<1 mm in length) the frequency of modulation may, in principle, be in the THz range provided that nonlinear materials may be found with sufficiently short relaxation times. The search for fast large nonlinearities is one of the main efforts in this area.

Conclusions

Nonlinear optics is proving valuable to the fields of nonlinear dynamics and deterministic chaos. On one hand, basically simple optical systems can be constructed exhibiting the most interesting classes of chaotic behaviour enriched by the possibility of a quantum description. On the other hand, lasers and related nonlinear optical devices have a large and growing technical application, and the understanding, control and possible exploitation of sources of instability in these systems has considerable practical importance.

Experimental findings are, in general, not yet sufficiently comprehensive to permit the quantitative analysis necessary to fully test the theoretical models. More carefully controlled experiments are forthcoming from which, along with time series, power spectra and identification of routes to chaos, embedding procedures may be implemented to the attractors describing the dynamical behaviour of these systems.

More detailed discussions of points in this review can be found elsewhere. For laser instabilities see refs 37-39 and also refs 40, 41 which cover both active and passive systems. For passive systems see ref. 42. Comprehensive treatments on the more general principles of deterministic chaos can be found in refs 3, 4. See the recent text on deterministic chaos by Schuster⁴³. See refs 8, 9, 44-46 for articles on the general aspects of deterministic chaos, and refs 47 and 48 for discussions on the device application of optical bistable systems.

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Observation of an unexplained event from a magnetic monopole detector

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A single event was recorded by our 0.18 m² inductive magnetic monopole detector, in a total effective observation time of 8,242 hours. None of the monitoring channels that record vibration, radiofrequency interference, and so on, indicated any extraneous cause for this event. An astrophysical estimate of the maximum number flux of monopoles suggests that the chances of a monopole having caused the event are less than 1 in 10³. Alternative explanations for the candidate are considered, but none of them seem likely.

GRAND unification theories (GUTs) suggest that massive magnetic monopoles (mass $\sim 10^{16}$ GeV) were created profusely during the very early stages of the formation of the Universe, while it was still extremely hot. These electrically neutral monopoles should carry magnetic charge h/e , as do the magnetic monopoles introduced by Dirac¹ more than 50 years ago, or perhaps a small integer multiple of the Dirac charge. The question of how many monopoles are still to be found is much less certain²; at one extreme, theories of inflation³ require that there is at most one monopole in our Universe. There is general agreement that any surviving GUTs monopoles should be moving rather slowly compared with the velocity of light, and consequently interact very weakly with matter; for this reason they are expected to be highly penetrating. The observation 4 years ago by Cabrera⁴ of a candidate cosmic magnetic monopole, using a small (10 cm²) inductive detector, stimulated considerable experimental and theoretical activity². An immediate problem was that Cabrera's candidate, if genuine, implied a number flux of monopoles far higher than astrophysical estimates appeared to allow. This 'Parker' bound⁵ arises from considering the outflow of energy from galactic magnetic fields to magnetic monopoles accelerated by passing through them, and comparing it with the rate at which the fields can be regenerated. Although there are some uncertainties⁶, the limit seems to be $\sim 10^{-15}$ cm⁻² sr⁻¹ s⁻¹, equivalent, on average, to ~ 1 monopole per 10⁶ yr through Cabrera's original detector.

Inductive detectors

The attractive feature of inductive detectors is that they are specific to magnetically-charged particles, and do not respond to electric charges or magnetic dipoles. A magnetic charge traversing a closed conducting loop induces a current, by Faraday's law of induction, that generates magnetic flux equal in magnitude to that carried by the magnetic charge. The magnetic flux associated with a Dirac monopole is 4.1×10^{-15} Wb, equal to $2\Phi_0$, where Φ_0 is the flux quantum of superconductivity. The size of the induced current is independent of monopole speed, and, for an isolated loop, of monopole trajectory. In practical detectors, the loop is made superconducting, so that the induced current is permanent and more easily measurable. The required current sensitivity of nA or better can be attained, non-dissipatively, with SQUIDS (superconducting quantum interference devices). One further use of superconductors in monopole detectors is the incorporation of a superconducting shield around the detector loops, which screens them from external field changes that would otherwise also induce currents. However, the presence of the shield complicates the response of the

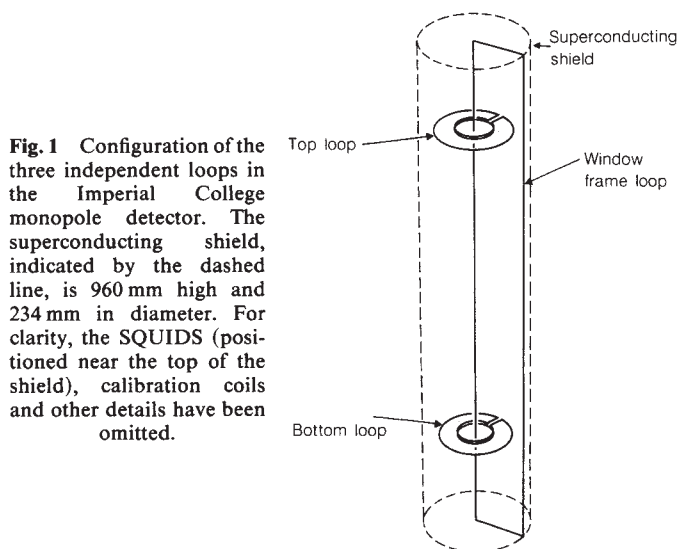


Fig. 1 Configuration of the three independent loops in the Imperial College monopole detector. The superconducting shield, indicated by the dashed line, is 960 mm high and 234 mm in diameter. For clarity, the SQUIDS (positioned near the top of the shield), calibration coils and other details have been omitted.

detector loop to a monopole: the size of induced current is then affected by trajectory, to a degree that depends on relative sizes of loop and shield. It is usual to include also some room temperature magnetic shielding (mu-metal or similar), to reduce the field acting on the superconducting shield.

The design of recent inductive searches⁷⁻¹⁰ (none of which reported any candidate monopoles) has been directed towards: (1) greatly enlarged detector area; (2) eliminating, or at least monitoring, as many as possible potential causes of spurious monopole-like events, and (3) the provision of multiple detector loops. Our detector^{9,11}, designed soon after Cabrera's original report, has three independent detector loops (Fig. 1). The two circular loops (the B and T loops) each has area averaged over 4π of 100 cm²; if the number flux of magnetic monopoles were to be, for some reason, many orders of magnitude above the Parker limit, the B and T loops would detect events (relatively) frequently. Also, the presence or absence of coincident signals in the B and T loops would provide a limited degree of directional information. The third 'window-frame' (WF) loop serves two purposes: any monopole passing through one of the circular loops would give a confirmatory coincident signal in the WF loop. The other role of the WF loop is to maximize the effective detector area within the available low-temperature and magnetically-shielded volume; it is arranged so that any monopole track through the superconducting shield, even one that has not intersected the WF loop itself, gives a signal. However, the signal

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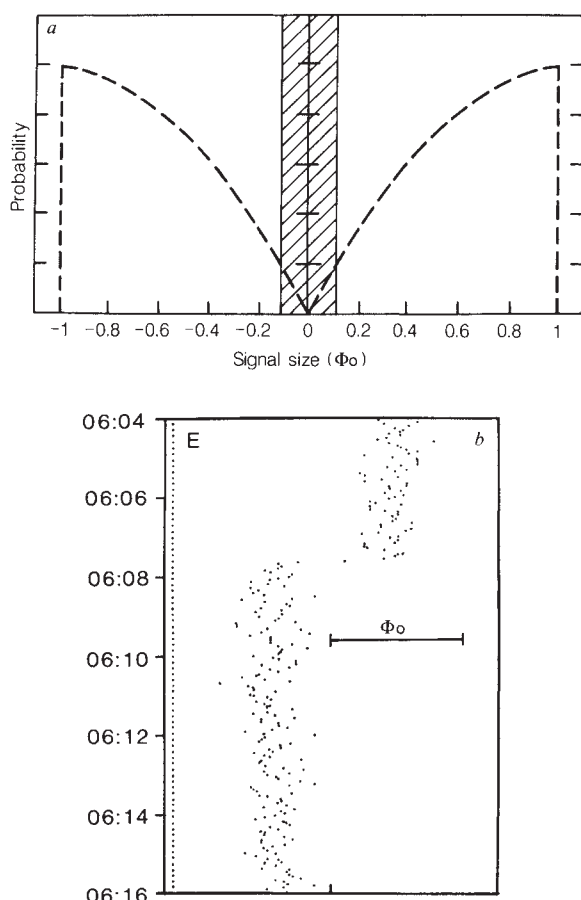


Fig. 2 *a*, The probability distribution of signal sizes in the WF loop, calculated for an isotropic influx of monopoles. Signals within the hatched region are too small to activate the event trigger. *b*, Section of the detailed high-frequency (20 samples per second) record, showing event-160 recorded at 07:06 h (BST) on 11 August 1985. Time is shown in minutes and seconds. The bar indicates the offset corresponding to a change in flux of Φ_0 .

size in the WF loop does depend on monopole trajectory, and ranges between 0 and $1 \Phi_0$ (Fig. 2*a*), and less than half of all trajectories give a measurable coincident signal in the B or T loops¹¹.

We anticipated three main causes of spurious events: mechanical shock, radiofrequency (r.f.) interference and magnetic field changes. As far as possible, the detector design incorporated isolation from these factors, and in addition we included relevant monitors (accelerometer, r.f. detector and magnetometer). The detector was tested with deliberate external disturbance, at levels of at least two orders of magnitude greater than ambient, and found to be totally insensitive to r.f. interference and changes in external magnetic field. However, it did respond to pressure fluctuations within the helium cryostat, arising from other users of the helium return line; isolation was improved and a pressure monitor added.

The detector loop currents are amplified with a bandwidth of 10 Hz, chosen so that the peak-to-peak noise approaches Φ_0 in magnitude, and thus maximizing the amount of significant information that is collected. A computer controlled data acquisition system (DAS) samples each detector and monitor channel 20 times per second. Shifts in any one of the detector outputs of more than a fraction of a Φ_0 occurring in a period of <100 s trigger storage of the data on floppy disc; this record covers 300 s before the trigger and 100 s after it. Digital filtering of the

recorded data allows the bandwidth to be narrowed to $\sim 10^{-2}$ Hz, and the r.m.s. noise to be reduced to $\sim 10^{-2} \Phi_0$. In addition, less detailed data, the maxima and minima in each channel for every 100-s period, are available as a permanent printed record. As a further precaution, the three detector outputs are recorded also on conventional chart recorders.

The detector became fully operational in September 1984, and we have reported previously⁹ on its first half-year of operation. During that period, there were about 110 putative events recorded by the DAS; most had immediate and obvious causes, such as low helium level, or cryostat pressure fluctuations. However, there were 12 'non-trivial' events, of which eight were in the T loop (almost certainly caused by a 'weak-link' in the superconducting circuit), three in the WF loop (associated with preceding mechanical shock), and one in the B loop (at a time when that channel was unstable); thus, detailed examination of the record allowed all 12 'non-trivial' events to be discarded as candidate monopoles⁹. The detector continued to run until 30 September 1985, accumulating a total of 8,242 h of active observation. In the second half-year, there were a further 60 putative events, but apart from the one event considered here, there were none in the 'non-trivial' category.

Detailed analysis of the total 170 putative events will be published elsewhere¹¹, but note that not one of them could be ascribed to two of the anticipated sources of interference: r.f. spikes and external magnetic field changes (r.f. interference in our laboratory is substantial, with capacitor bank discharges in nearby rooms, and a powerful short-wave transmitter at a distance of 50 m; the local magnetic field fluctuates by about 10^{-6} T, with the largest contribution from nearby lifts). This immunity is consistent with the tests on the detector mentioned above. In July 1985, the magnetometer became needed urgently elsewhere, and was removed from the experiment.

11 August 1985 event

At 07:06 h BST on Sunday 11 August 1985, event-160 occurred (Fig. 2*b*), of magnitude $0.83 \pm 0.04 \Phi_0$ (the uncertainty is primarily that in the calibration of the detector loop), close to the maximum in the calculated distribution of signal sizes in this loop (Fig. 2*a*).

At the time of event-160, the two other detector channels, the B and T loops, showed no obvious coincident offsets; detailed analysis of the data puts limits of 0.01 ± 0.04 (standard deviation, s.d.) and 0.02 ± 0.06 (s.d.) Φ_0 on the signals in those channels. The noise levels in all three detector channels had their usual magnitudes, and application of a range of statistical tests to the data revealed no anomalies. Nor was any obvious abnormal behaviour to be seen in the channels monitoring vibration, r.f. interference and cryostat pressure.

Inspection of the less detailed monitoring record and of the conventional chart records for the days before and after event-160 revealed nothing unusual. The weekly liquid helium refill had been on 6 August, with a moderately noisy period on 7–8 August which always occurs after a refill, and is probably caused by differential thermal expansion within the cryostat. From 11.00 h on 10 August to 09.00 h on 12 August conditions were very quiet, but not unusually so for a weekend. As soon as possible after the event occurred, the detector system, including the monitoring devices, was checked thoroughly for normal operation.

The detector was allowed to continue running normally until 30 September, and then subjected, while still at helium temperatures, to tests. Finally, it was warmed slowly to room temperature, so that an internal inspection could be made.

Later, detailed statistical analysis of the data for event-160 turned up one feature that had no immediate explanation: in quiet (night-time and weekend) conditions the output of the r.f. monitor has a characteristic pattern, with a mean of 5V and peak-to-peak fluctuations of 2V. The r.f. record for event-160 showed the usual pattern, but superimposed on it was a small,

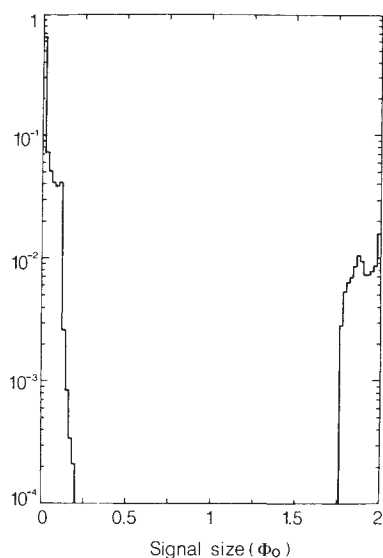


Fig. 3 The probability distribution of signal sizes in the B and T loops, calculated for those monopole trajectories, selected from an isotropic influx, that give a WF signal in the range $0.78\text{--}0.88 \Phi_0$. Positive and negative signals are equally likely.

though statistically highly significant, increase of 100 mV in the mean level, coincident in time with the event itself. We believe this shift to be a consequence of the offset in the WF channel, and it is, therefore, simply an instrumental artefact. The essence of the mechanism linking the two channels is as follows: in quiet conditions, $\sim 90\%$ of the recorded r.f. interference is self-generated, by the digital pulses transmitted between the DAS and its controlling computer. Thus, the pattern of binary digits representing the signals in the various channels is itself the prime determinant of the r.f. level. Event-160 caused a sign change in the output of the WF loop, and the sign is read by the DAS as the most significant bit. The sign bit was always positive before the event, and negative afterwards, and thereby altered the radiated r.f. interference in a systematic fashion. In practice, the mechanism so far described is complicated by the presence in the r.f. monitor of a peak sample-and-hold amplifier with 0.1 s time constant (its function is to ensure that fast r.f. spikes are recorded properly), so that there is in effect a convolution of the r.f. radiated over a period of a few tenths of a second. This complication removes the straight one-to-one relationship between the digit pattern in the WF channel, say, and the recorded r.f. level. However, a simple statistical test on the data for event-160 demonstrated that such an effect was present: because the magnetometer was at that time disconnected, its channel to the DAS was digitized as a small number fluctuating about zero. A correlation with the r.f. channel showed that the consequent alternation of sign sometimes produced changes in the r.f. level of ~ 150 mV in the correct sense. That the effect was not always present, and that when it was there it gave an offset slightly larger than the 100 mV increase associated with event-160, is understandable in terms of the effect of the peak sample-and-hold amplifier. An experimental test was then made in quiet nighttime conditions, using a square-wave oscillator at about 0.5 Hz connected to the WF channel of the DAS. Again, a response in the r.f. channel could be produced.

Thus all our checks show that event-160 took place at a time when the detector was functioning properly, and in the absence of any indication of external interference.

The record of event-160 meets the 'risetime' criterion that can be imposed: relic monopoles, if they exist, are believed to be moving rather slowly, $v/c \sim 10^{-3}$, but it is unlikely that their

speed is orders of magnitude less than that². Consequently, the transit time of a monopole through our detector should be of the order of microseconds. The 10-Hz bandwidth of the amplified detector loop outputs is equivalent to a 65 ms risetime (0–90%) for a step input; the record of event-160 shows that its risetime is less than two sampling periods (100 ms), and is certainly consistent with a step input, as would be given by a monopole.

We have calculated the distribution of event sizes in the B and T channels for monopole trajectories giving WF signals in the range $0.78\text{--}0.88 \Phi_0$ (Fig. 3). About 70% of such tracks give signals that are $< 0.1 \Phi_0$, which would be below the noise level, so that the absence of a signal in either the B or T channels coincident with event-160 in the WF channel is consistent with that event being caused by a monopole. Furthermore, if some signal of improbable size had been seen in either of those two channels, it would have indicated the presence of a spurious cause for event-160.

Possible explanations

Unauthorized interference. Appropriately sized offsets can be induced manually, by adjusting the SQUID control unit, but our attempts to do so were not fast enough to be recorded as risetime limited events. Also, tests showed that it was practically impossible to enter and leave the laboratory without there being some trace on the accelerometer. On these two counts, we believe that this explanation can be discounted.

Electronic fault. An offset generated in the WF loop SQUID control unit could mimic a monopole. On one occasion we have had such a fault, which produced temporary offsets of $\sim 20 \Phi_0$ that lasted for a few minutes, and recurred several times in a month. Faults of this kind could have any size up to $\sim 10^3 \Phi_0$, and would be likely to recur, particularly if the control unit is given some mechanical shock. After event-160, none of these symptoms were apparent. At the end of our observation period, all three SQUID control units were examined for evidence of electrical and mechanical failure. None was found, apart from some mild corrosion on a printed circuit board in the WF unit; that particular board was remote from the section of the unit that could easily produce offsets.

A fault in the data acquisition system can be excluded because of the 'back-up' chart recorder which showed an appropriate offset corresponding to event-160.

Mechanical shock. As described previously⁹, we have seen several events, particularly in the WF loop, that have been induced by mechanical shock; sometimes these have been accompanied by small offsets in the B loop. The mechanism for this response to shock is, we believe, small movements and distortions of the detector loop assembly within the superconducting shield, which alter slightly the coupling of residual flux into the loops; the design of the B and T loops makes them intrinsically less sensitive than the WF loop to this effect.

The accelerometer is mounted at the top of the cryostat, and responds to accelerations in a single direction (in directions perpendicular to its axis, the sensor response is down by about two orders of magnitude). Consequently, no quantitative correlation can be expected between the size of an event induced by this mechanism, and the recorded shock amplitude. However, we found a well-defined trend linking the two (Fig. 4).

Throughout the 400 s detailed record of event-160, the vibration level was $< 5 \times 10^{-4} g$, and for the previous 20 h had not exceeded $2 \times 10^{-3} g$ (weekday peak levels are up to $10^{-1} g$). The low level of vibration at the time of event-160, and for an extended period before and after, sets it well away from the trend of Fig. 4, suggesting that the event was not caused by mechanical shock.

Motion of trapped flux in the shield. When it was cooled down, the superconducting shield surrounding the detector loops trapped the residual magnetic flux, estimated to correspond to a field of about $10^{-9} T$ (the field inside the room temperature

magnetic shields). In principle, this flux is thermodynamically metastable, and if it were to move, it could simulate the passage of a monopole. Because the WF loop is coupled strongly to the shield, whereas the B and T loops are effectively decoupled, such flux motion would be likely to generate a larger signal in the WF loop than in the other two channels.

Movement of pinned flux can be activated thermally (although it may be possible to induce motion with mechanical shock, and in other ways), and so after the initial cooldown we followed the standard procedure of 'annealing' the shield, by maintaining it for some time above its usual operating temperature. Since then, there have been several occasions on which refilling with liquid helium has been delayed, and in consequence the shield has warmed slightly. Thus, any flux that was prone to move would most probably have done so during those periods. Also, the probability of flux depinning declines slowly (logarithmically¹²) with time.

Our detector is certainly much less stable when the helium level is low (and we, therefore, exclude such periods from the total of active observation time), but the data indicate that causes other than flux motion in the shield, such as mechanical movement arising from thermal expansion, are the main culprits. In any case, in those periods of low helium level, the offsets in the WF channel were usually $<0.2 \Phi_0$; on just one occasion there was an offset of $0.4 \Phi_0$.

Thus, even when the superconducting shield, which is made from 3-mm thick lead, is warmer than usual, we have seen no clear-cut evidence of flux moving in it. Our experience is similar to that of others (B. Cabrera, and H. Frisch, private communications), that low densities of trapped flux in thick superconducting shields are extremely stable.

While we cannot exclude flux motion in the shield as the cause of event-160, the empirical evidence is that it is unlikely. **Flux motion in SQUID sensor.** The SQUID sensors are fabricated from blocks of superconductor (niobium), so that they also trap metastable magnetic flux.

Experiments have been carried out on SQUID sensors with short-circuited input terminals (so as to avoid confusion with

effects generated external to the SQUID)¹³, and it has been demonstrated that mechanical shock, either from the outside or internal shocks from thermal expansion, can induce offsets. These have been interpreted as arising from flux motion within the SQUID. However, those experiments were carried out in trapped fields of $\sim 10^{-4}$ T (ambient field), whereas our sensors are within the magnetic shields and in a field of only $\sim 10^{-9}$ T. There is some evidence (B. Cabrera, personal communication) that such offsets are larger and more frequent the higher the field.

In both the Stanford⁷ and the Chicago⁸ inductive detectors several offsets were seen, mostly soon after initial cool down, that were attributed tentatively to this cause (the absence of coincidence and also the signal sizes excluded those events as candidate monopoles). However, in those two experiments, the fields at the SQUIDs are respectively 2 and 3 orders of magnitude greater than in our detector, making them substantially more sensitive to this problem.

The evidence from our experiment indicates that mechanical shock from whatever source affects primarily the detector loops, rather than the SQUID sensors themselves: the WF channel responds most to mechanical shock, with occasional associated smaller offsets in the B and T channels; if it were the SQUID sensors that were being affected, we would expect to see uncorrelated offsets occurring at random in all three channels. As at Stanford and Chicago, our detector was much noisier in the first few days after initial cooldown (for example, events 0, 1 and 16, Fig. 4), and we believe that this was associated with relaxation of mechanical strain, in both the detector assembly and the cryostat, rather than in the SQUID sensors. The evidence of a year's experience with the detector, coupled with the reported behaviour of other experiments, gives us no reason to attribute event-160 to flux motion within the SQUID sensor.

Inadvertent excitation of calibration coils. Each detector loop is fitted with a toroidal calibration coil which can be energized externally. Event-160 could be simulated by a small current, of the order of 100 nA, being induced in the WF calibration coil. For example, if the insulation between the leads to the coil had broken down, thermoelectric voltages, which are invariably present at the microvolt level, could have generated sufficient current. When checked later, the insulation to these coils was in perfect condition, so we can exclude this possibility.

Mechanical fault in detector. When all other checks had been completed, the detector was warmed slowly to room temperature and examined carefully. Some small lead shavings, typically 1-mm in size, were found on the detector loop formers. Presumably, when the detector was originally assembled, and the close-fitting lead shield was pulled over the formers, these particles were scraped off. Because the particles would be quite strongly diamagnetic when superconducting, the flux coupled into a detector loop would alter if a particle were to move. However, an order of magnitude calculation shows that even in the worst possible case, with the particle adjacent to the wire of the detector loop, the particles would need to be at least 10^2 larger in volume to give an offset of the size required. Consequently, we can rule out this mechanism.

In all other respects, the detector assembly was found to be in pristine condition.

Cosmic rays. We consider the likely effect of cosmic ray radiation on both the detector electronics and the SQUID sensor. At sea level, the total flux of neutrons produced by cosmic-ray primaries is $\sim 4 \times 10^{-2} \text{ cm}^{-2} \text{ s}^{-1}$ for energies $>100 \text{ keV}$, and $\sim 3 \times 10^{-3} \text{ cm}^{-2} \text{ s}^{-1}$ for energies above 50 MeV (ref. 14); the proton fluxes at those energies are much lower. In the interaction of cosmic ray particles with solid state electronics, the reaction n (or p) + Si $\rightarrow \alpha$ + heavy nucleus is thought to be the important mechanism¹⁴. Both reaction products can generate large numbers of electrons, up to 10^6 , by ionization, leading to 'soft errors' (spontaneous changes in logic state) in memory circuits. Recently, Nicholls *et al.*¹⁵ have found that, as the incident

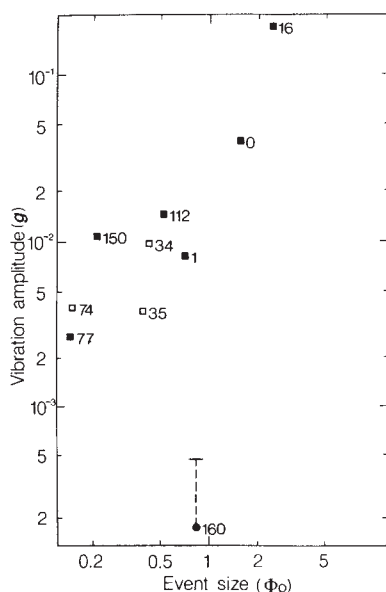


Fig. 4 Correlation between mechanical shock and event size in the WF channel. The shock is that measured at the top of the cryostat and the events are numbered in chronological order. ■, Events with simultaneous (within the 50-ms time resolution of the data acquisition system) shock and event; □, events with preceding shocks, as discussed in ref. 9. ●, Event-160 (the attached bar indicates the maximum shock during the entire 400 s detailed record of the event).

particle energy increases from 20 to 50 MeV, the soft error rate in a variety of devices rises sharply.

Ziegler *et al.*¹⁶ have subjected a small superconducting inductive magnetic monopole detector (with identical SQUID electronics to ours) to bombardment with neutrons of energies 10–16 MeV, and a total dose of 8.6×10^8 neutrons cm^{-2} (equivalent to seven centuries of cosmic-ray dose), and observed no offsets in the detector output. However, in the light of Nicholls *et al.*'s most recent data on energy dependence¹⁵, Ziegler has suggested (personal communication) that their experiment on a monopole detector should be repeated at higher neutron energies.

Our detector would have been subjected to a total flux of cosmic-ray neutrons with energies above 50 MeV of $\sim 10^5 \text{ cm}^{-2}$. The active area of semiconductor devices in significant sections of the circuitry is not more than 10^{-1} cm^2 ; in Si about $10^{-6} \alpha$ particles are generated per cosmic-ray neutron¹⁴, so we estimate the probability of a soft error type of charge burst as $\sim 10^{-2} \text{ y}^{-1}$. However, the mechanism by which such an event might affect the SQUID electronics is unclear: any change in the SQUID control logic circuitry would almost certainly lead to offsets orders of magnitude larger than event-160. Another possibility is that defects created during a soft error type of event could change sufficiently the operating point of a device in the analogue section of the circuitry, and so induce an offset.

The behaviour of Josephson junctions under α -irradiation has been studied also¹⁷. Near the critical current of the junctions, the 'upset' rate per α particle approaches unity. The rate at which α particles are produced by cosmic-ray neutron bombardment is not strongly target dependent, so we may estimate the α -particle flux (generated by neutrons with energies above

50 MeV) incident on our SQUID sensors as $0.3 \text{ cm}^{-2} \text{ yr}^{-1}$. The SQUID weak link has area $\sim 10^{-6} \text{ cm}^2$, so that the probability of a cosmic-ray induced SQUID offset is no more than 10^{-6} yr^{-1} . Were such an offset to occur, we would again expect it to be much larger than event-160.

Although the probabilities of these cosmic-ray mechanisms are low, they are finite and cannot be discounted entirely.

Conclusions

So far, we have no obvious explanation for event-160. The total area-time product of our experiment corresponds to 400 'Cabreras', the unit of exposure equivalent to that of Cabrera's original experiment. The exposure of the three other large detectors now totals 800 'Cabreras' (refs 7, 8, 10 and B. Cabrera, personal communication), so that of itself, our candidate is not statistically at odds with the absence of events in the other experiments. At 90% confidence level, the three other experiments taken together give an upper bound on the cosmic monopole flux of $2 \times 10^{-12} \text{ cm}^{-2} \text{ sr}^{-1} \text{ s}^{-1}$; if our experiment and its candidate event are included, there is a lower bound (at 90% confidence level) on the flux of $5 \times 10^{-14} \text{ cm}^{-2} \text{ sr}^{-1} \text{ s}^{-1}$, which is still two orders of magnitude above the Parker limit. None of the alternative explanations that we have discussed here for event-160 seem particularly likely, and it is clearly possible that some yet unidentified cause was responsible for it. The results from the larger inductive detectors that are now under construction should help to resolve the puzzle.

We thank A. Cayless, A. Chhabra, W. G. Jones, D. Podger, W. S. Steer and D. Websdale for assistance. Financial support was received from the Particle Physics Committee of SERC and from Imperial College.

Received 6 February; accepted 26 March 1986.

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Molecular distinction between fetal and adult forms of muscle acetylcholine receptor

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Distinct classes of acetylcholine receptor channels are formed when Xenopus oocytes are injected with combinations of the bovine α -, β -, γ - and δ - or the α -, β -, γ - and ϵ -subunit-specific messenger RNAs. The conductance and gating properties of the two classes of channels, in conjunction with the developmental changes in the muscular contents of the mRNAs, suggest that replacement of the γ -subunit by the ϵ -subunit is responsible for the functional alteration of the receptor during muscle development.

DIFFERENT forms of the nicotinic acetylcholine receptor (AChR) occur in mammalian skeletal muscle during development. The AChR is present predominantly at the neuromuscular junction in adult muscle, whereas in fetal muscle, as well as in denervated adult muscle, the AChR is also found in the non-

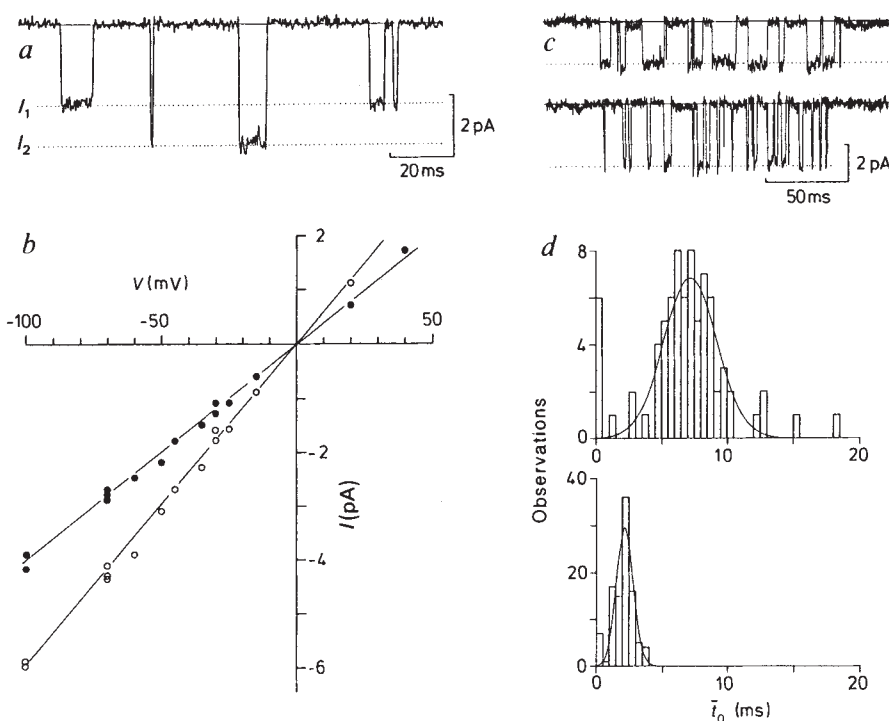
junctional membrane. The junctional and non-junctional types of AChR channels differ in conductance and gating properties^{1–3}. During formation of the neuromuscular contact, two classes of AChR channels coexist in the same muscle fibre^{4–7}. Some reports suggest that the different forms of the AChR are

Fig. 1 Properties of ACh-activated single-channel currents in oocytes injected with the calf AChR α -, β -, γ -, δ - and ϵ -subunit-specific mRNAs. **a**, Single-channel currents activated by 0.5 μ M ACh. Outside-out patch. Inward current downwards. Membrane potential, -60 mV. Low-pass filtering at 1 kHz (-3 dB). The short interruptions of the elementary currents occurring in the time range of tens of microseconds are largely attenuated. Two classes of elementary currents are observed and are marked by I_1 and I_2 (dotted lines). **b**, Single-channel I - V relations. Each point is the mean amplitude of at least 20 elementary currents. Data from four outside-out patches. Straight lines are linear regression lines and indicate channel conductances of 40 pS (filled symbols) and 60 pS (open symbols). Interpolated current reversal potentials are 0.6 mV and 0.5 mV, respectively. **c**, Bursts of single-channel currents activated by 10 μ M ACh. Cell-attached patch recording. Two bursts recorded on the same patch are shown. Note that current pulses in the two bursts have different mean amplitudes of 2.3 pA and 3.4 pA. Membrane potential of -70 mV was estimated using single-channel conductances of 40 pS and 60 pS and the mean reversal potential of single-channel currents. In three experiments, the oocyte resting membrane potential was measured during patch current recording using an intracellular microelectrode. The reversal potential determined by interpolation of inward and outward currents was -10 ± 2 mV. **d**, Distribution histograms for the average durations of elementary currents during single bursts. Each entry represents the mean of the durations of all elementary currents occurring in a single burst (t_0). Upper graph represents measurements on 40-pS conductance channel (77 bursts), lower graph on 60-pS conductance channel (101 bursts). Continuous lines are single gaussians. The left-most bins in both graphs and the two right-most bins in the upper graph were not taken into account. Means of the two distributions are 7.2 ± 2.0 ms ($n = 69$) and 2.3 ± 0.6 ms ($n = 94$). Cell-attached patch. Estimated membrane potential was -70 mV (that is, -60 mV driving potential). A burst was defined as any sequence of elementary currents separated by a closed interval longer than 50 ms. This value was chosen after inspection of the distribution of the closed intervals of the whole current record. It was characterized in both cases by a short component (5.4 ms and 6.4 ms average duration, >95% of the total area of the distribution in both cases) and two much longer components with durations of several hundreds of milliseconds. Bursts consisting of less than five current pulses were excluded.

Methods. The calf AChR α -, β -, γ -, δ - and ϵ -subunit-specific mRNAs were synthesized *in vitro*^{25,28}, combined in a molar ratio of 2:1:1:1:1 and injected into *Xenopus laevis* oocytes (total mRNA concentration, 160 ng μ l⁻¹; average volume injected per oocyte, ~50 nl). Whole-cell current measurements^{27,28} were made on oocytes after incubation²⁶ for 2-3 days. Single-channel current measurements were made on oocytes after 2-5 days' incubation followed by removal of the follicular cell layer and the vitelline membrane^{28,36}. All single-channel current recordings were carried out at 21 ± 1 °C. In experiments with outside-out patches, pipettes²⁸ were filled with a solution of the following composition (in mM): KCl, 20; KF, 80; MgCl₂, 1; EGTA, 10; HEPES (pH 7.2), 10. The extracellular solution had the composition (in mM): NaCl, 115; KCl, 2.5; CaCl₂, 1.8; HEPES (pH 7.2), 10. Patch membrane potentials of outside-out patches were obtained by correcting the pipette potential by -5 mV for liquid junction potentials³⁷. In records from cell-attached patches, the pipette was filled with extracellular solution with ACh added at 0.5-1 μ M or 10 μ M. Patch-clamp currents were stored on magnetic tape, filtered at 1 kHz (-3 dB), digitized at 0.2-ms intervals and analysed in a PDP 11/73 computer by a semi-automatic procedure to compute distributions of current amplitudes and durations³⁸. Distributions of amplitudes were fitted with one or two gaussians and distributions of durations by one or two exponential components; those with two components were made by maximizing the likelihood function³⁸.

structurally distinct⁸. The mammalian muscle AChR is thought to have a subunit structure of $\alpha_2\beta\gamma\delta$, as is the case for the *Torpedo* electroplax AChR⁹⁻¹¹. The primary structures of all four subunits of fish¹²⁻¹⁷ and mammalian AChRs¹⁸⁻²⁴ have been elucidated by recombinant DNA techniques. In addition, a novel subunit, termed the ϵ -subunit, has been discovered by cloning and sequencing the DNA complementary to the calf muscle messenger RNA encoding it²⁵. cDNA expression studies with *Xenopus* oocytes²⁵⁻²⁷ have indicated that the calf ϵ -subunit, which shows higher sequence homology with the γ -subunit than with any other subunit, can replace the *Torpedo* γ -subunit to form the functional receptor in combination with the *Torpedo* α -, β - and δ -subunits. Single-channel current measurements have indicated that hybrid AChRs composed of subunits from the two species form channels possessing different gating behaviour²⁸. These findings raise the intriguing possibility that the different forms of the AChR observed during muscle development may arise from different combinations of the subunits.

One approach to this issue would be to produce AChR channels in *Xenopus* oocytes by microinjection of various combinations of the subunit-specific mRNAs and to compare the conductance and gating properties of these channels with those of native AChR channels in fetal and adult muscle. We report here that oocytes injected with mRNAs specific for the α -, β -, γ -, δ - and ϵ -subunits of the calf muscle AChR yield two distinct classes

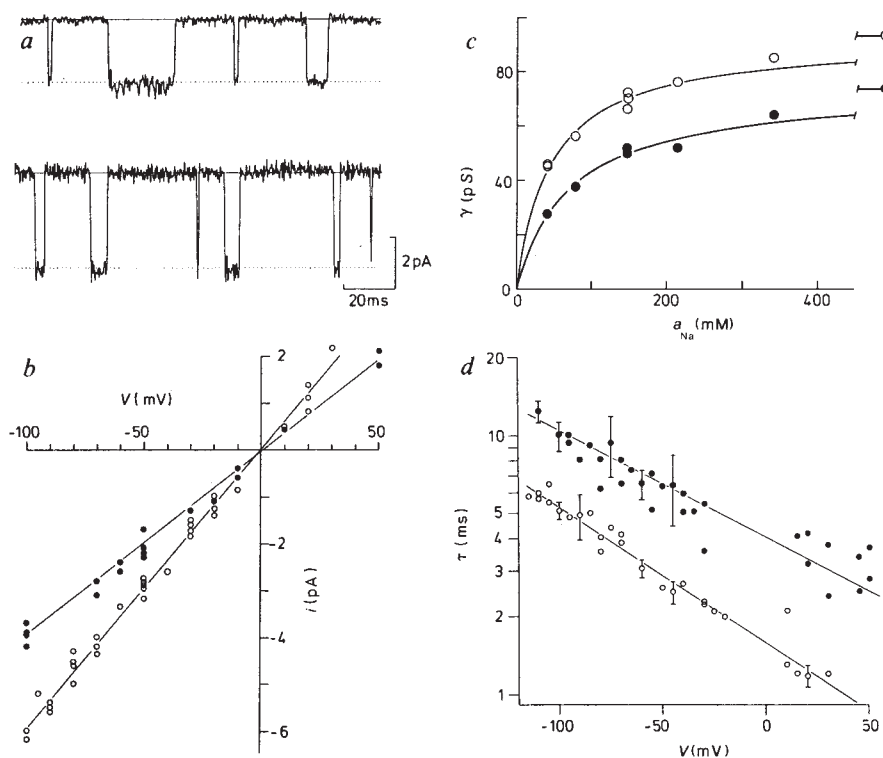


of AChR channels. In contrast, only one of these two channel classes is formed in oocytes injected either with the α -, β -, γ - and δ -subunit-specific mRNAs or with the α -, β -, δ - and ϵ -subunit-specific mRNAs. The two classes of channels, designated AChR _{γ} and AChR _{ϵ} respectively, differ in conductance and gating behaviour; AChR _{γ} is similar in single-channel properties to the AChR in fetal calf muscle, whereas AChR _{ϵ} resembles the AChR in adult bovine muscle. This finding, together with the developmental changes observed in the contents of the γ - and ϵ -subunit mRNAs in bovine muscle, suggests that the different properties of the fetal and adult muscle AChRs are a result of the γ -subunit being replaced by the ϵ -subunit during muscle development.

AChR channels from five subunits

The mRNAs specific for the α -, β -, γ -, δ - and ϵ -subunits of the calf muscle AChR were synthesized by transcription *in vitro* of the respective cDNAs as described previously^{25,28}. The five subunit-specific mRNAs were combined and injected into *Xenopus* oocytes. Figure 1a shows a record of acetylcholine (ACh)-activated single-channel currents from an outside-out membrane patch isolated from an oocyte implanted with the five AChR subunits. The distribution of current amplitudes is characterized by two well-separated peaks of mean amplitude 2.6 pA and 3.9 pA (at -60 mV membrane potential). The single-channel current-voltage (I - V) relations of both classes of

Fig. 2 Properties of ACh-activated single-channel currents in oocytes injected with the α -, β -, γ - and δ -subunit-specific mRNAs (AChR _{γ}) or with the α -, β -, δ - and ϵ -subunit-specific mRNAs (AChR _{ϵ}). **a**, Single-channel currents activated by 0.5 μ M ACh. Outside-out patches. Inward current downwards. Upper trace, recording from an oocyte with AChR _{γ} . Mean current amplitude, 2.4 pA. Lower trace, recording from an oocyte with AChR _{ϵ} . Mean current amplitude, 3.6 pA. Membrane potential, -60 mV in both experiments. Low-pass filtering at 1 kHz (-3 dB). **b**, Single-channel I - V relations of the AChR _{γ} (filled symbols, 7 outside-out patches) and the AChR _{ϵ} channel (open symbols, 6 outside-out patches). Straight lines are linear regression lines. Their slopes are 39 pS and 59 pS for the AChR _{γ} and AChR _{ϵ} channels, respectively. Interpolated current reversal potentials are 1.3 mV and -0.2 mV. **c**, Conductance (γ) versus Na⁺ activity (a_{Na}) for AChR _{γ} (filled symbols) and AChR _{ϵ} (open symbols). Slope conductance was measured at -20 to -100 mV driving potential on outside-out patches. The I - V relations in this region were fitted by linear regression lines. The pipette solution was as described in Fig. 1 legend. The extracellular solution contained 50-500 mM Na⁺ and 1.8 mM CaCl₂. The Na⁺ activities are those for pure NaCl solutions³⁹. No correction for the differences in osmolarity of various extracellular solutions was made. Continuous lines represent hyperbolas according to $\gamma = \gamma_{\text{max}} a_{\text{Na}} / (a_{\text{Na}} + K_m)$, where γ_{max} is the limiting conductance and K_m is the Na⁺ activity at which the channel conductance is half-maximal. Limiting conductances of 75 pS for AChR _{γ} and 93 pS for AChR _{ϵ} , obtained by a nonlinear least-squares fit, are indicated by the symbols on the right. Half-saturating Na⁺ activities were 73 mM for AChR _{γ} and 46 mM for AChR _{ϵ} . **d**, Average duration (τ) of elementary currents versus membrane potential in semilogarithmic coordinates. Filled symbols, AChR _{γ} channel; open symbols, AChR _{ϵ} channel. Data are from seven (filled symbols) and six (open symbols) cell-attached patch recordings. Each point represents a determination of τ from the distribution of single-channel currents or from noise analysis of the single-channel current record (see Methods). Bars indicate 1 s.d. from 3-7 patches. Lines represent linear regression fits as specified in Table 1.



Methods. To avoid slight contamination by aberrant AChR channels resulting from injection of the α -, β - and δ -subunit-specific mRNAs (see text), oocytes were injected either with the α -, β -, γ - and δ -subunit-specific mRNAs or with the α -, β -, ϵ - and δ -subunit-specific mRNAs in a molar ratio of 2:1:10:1 (total mRNA concentration, 6.4 ng μ l⁻¹; average volume injected per oocyte, ~50 nl). Patch current recording as described in Fig. 1 legend. In cell-attached recordings, the oocyte membrane potential was usually measured with an intracellular microelectrode (filled with 3 M KCl, 10-20 MΩ d.c. resistance) during the patch current recording. In some experiments, the oocyte membrane potential was estimated from the channel slope conductance and an assumed reversal potential of -10 mV. The average slope conductances were 40 ± 1 pS ($n=9$) for the AChR _{γ} and 59 ± 2 pS ($n=7$) for the AChR _{ϵ} channel. Current reversal potentials from whole-cell I - V relations were -11 ± 2 mV ($n=5$) for the AChR _{γ} and -9 ± 4 mV ($n=3$) for the AChR _{ϵ} channel. Current reversal potentials from single-channel I - V relations were -9 mV and -10 mV (mean of two patches) for the two channels. The average duration of elementary currents was estimated in two ways. In recordings with low channel opening frequency, where few superpositions (<5%) of elementary currents occurred, τ was estimated as the longer of the decay constants fitted to the distribution of current durations²⁸. In recordings where elementary currents superimposed more frequently, τ was estimated using standard techniques of noise analysis³¹. Segments of current record were low-pass filtered (0.8 kHz, 4-pole Butterworth) and digitized at 0.5- or 1-ms intervals. Power spectral densities were calculated on 512-point data sections by an integer Fast Fourier Transform routine on a PDP 11/34 computer; 30-80 such spectra were averaged. For the single-channel records close to the reversal potential, control spectra obtained at the current reversal potential were subtracted. Single lorentzians were fitted by eye to the difference spectra. τ was calculated from the corner frequency, f_c , of the lorentzian as $\tau = 1/(2\pi f_c)$.

currents were linear, and their reversal potentials were close to 0 mV (Fig. 1b). These observations suggest that the two classes of ACh-activated currents reflect the opening of two distinct AChR channels coexisting in the oocyte membrane. Their conductances were 40 ± 1 pS and 60 ± 2 pS (mean $\pm$ s.d., $n=4$).

The two classes of currents were clearly distinguishable from each other when activated by desensitizing concentrations of ACh (>5 μ M). Figure 1c shows two examples of ACh-activated currents recorded from the same cell-attached membrane patch in the presence of 10 μ M ACh. The currents occurred in bursts that lasted for hundreds of milliseconds and were separated by silent intervals of much longer duration. This pattern is comparable to that of ACh-activated currents observed in frog and rat muscle at high ACh concentrations (>5 μ M)^{29,30}. It reflects fluctuations of single AChR channels between long-lived inactive states (inter-burst intervals) and active states (bursts) where a given channel switches repeatedly between open and closed states. Within a given burst, all resolved elementary currents had a similar amplitude. However, bursts fell into two classes with respect to current amplitude. The average sizes of elementary currents in 178 bursts, measured in this patch at about -60 mV driving potential, were 2.3 pA and 3.4 pA, respectively, corresponding to the two current amplitudes observed at low ACh concentrations. A similar separation of bursts into two classes of amplitudes was observed in nine other patches

examined at high ACh concentrations, the average ratio of large to small current amplitudes being 1.5 ± 0.04 ($n=10$). The occurrence of two separate classes of bursts thus indicates that two populations of AChR channels characterized by differing conductances were formed in oocytes injected with the five subunit-specific mRNAs.

The relative frequency of occurrence of each class of currents varied with different oocytes and with different patches from a given oocyte. In every patch, however, the 40-pS conductance channel was observed more frequently than the 60-pS conductance channel. In records from 10 patches, 73 $\pm$ 10% of all currents were openings of the 40-pS conductance channel. This may be due to a higher density of the 40-pS conductance channel or to different activation kinetics of the two AChRs.

The two classes of AChR channels also differ in their gating properties. Figure 1d shows the distributions of average durations of elementary currents measured from one patch during bursts of the 40-pS (upper graph) and the 60-pS conductance channel (lower graph). The average duration was 7.2 ms for the 40-pS conductance channel and 2.3 ms for the 60-pS conductance channel.

AChR channels from four subunits

Oocytes were injected with various combinations of four of the five subunit-specific mRNAs and then tested for response to

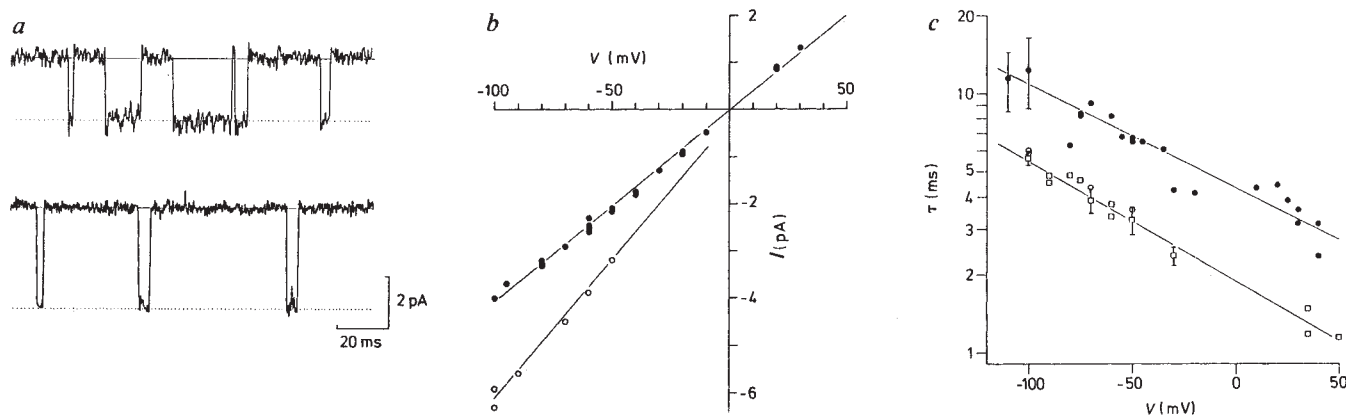


Fig. 3 Properties of ACh-activated single-channel currents in fetal and adult bovine muscle. **a**, Single-channel currents activated by $1 \mu\text{M}$ ACh in fetal (upper trace) and adult (lower trace) muscle. Outside-out patches. Inward current downwards. Membrane potential, -60 mV . Low-pass filtering at 1 kHz (-3 dB). Mean current amplitudes are 2.5 pA and 3.9 pA . **b**, Single-channel $I-V$ relations of ACh-activated currents in fetal (filled symbols, four outside-out patches) and adult (open symbols, two outside-out patches) muscle. The regression lines indicate slope conductances of 40 pS and 59 pS for AChR channels in fetal and adult muscle, respectively. Interpolated current reversal potential is 1.2 mV for fetal muscle. Extrapolated reversal potential is 7 mV for adult muscle. **c**, Average duration of elementary currents against membrane potential in semilogarithmic coordinates from fetal (filled symbols) and adult (open symbols) muscle. In fetal muscle, the τ values were derived from four cell-attached patches as described in Fig. 2 legend. In adult muscle, the τ values were estimated from distribution of single-channel current durations (two outside-out patches, open circles) or from the m.e.p.c. decay time constant (four endplates, open squares). Bars indicate 1 s.d. from three or four different experiments. Lines represent linear regression fits as specified in Table 1.

Methods. Recordings of ACh-activated currents through fetal calf AChR channels were made on myoballs maintained in cell culture. Muscle tissue from the leg of a calf fetus (3–4 months gestation), obtained from a local abattoir, was minced, plated and maintained in cell culture as described for fetal rat muscle⁴⁰. After 4–5 days, elongated myotubes had formed and were exposed to colchicine (10^{-7} M) for 12 h, resulting in the formation of rounded myoballs. All experiments were done 5–9 days after plating. Outside-out patches were isolated from myoballs using conventional patch pipettes containing either the same solution as described in Fig. 1 legend or with K^+ replaced by Na^+ . This was necessary in experiments on myoballs maintained in culture for more than 6 days because outward currents carried by K^+ were activated at membrane potentials more positive than -60 mV . The membrane potential in cell-attached recordings was estimated from the slope conductance ($40 \pm 2 \text{ pS}$, $n = 4$), assuming a current reversal potential of -10 mV . ACh-activated single-channel currents in adult bovine muscle were measured on sacro-coccygeal tail muscle of cows (*Bos taurus*, 2–5 yr old) at $20 \pm 1^\circ \text{C}$. Muscles were removed from the region of the last 10 coccygeal vertebrae (S10–S18)⁴¹ and consisted of many bundles of 50–100 fibres. Such bundles were dissected to 'sheets' of 10–20 fibres each and incubated with 1 mg ml^{-1} collagenase (Sigma type I) at $18\text{--}22^\circ \text{C}$ for 3–4 h in Ringer's solution of the following composition (in mM): NaCl, 140; KCl, 4; CaCl_2 , 1; MgCl_2 , 1; HEPES (pH 7.2), 10. This was followed by a 10-min exposure to Ringer's solution containing 0.05 mg ml^{-1} protease (Sigma type VII). Myelinated nerves and nerve terminals still attached to the endplate were then visible in edge fibres viewed with Nomarski optics ($\times 16$ objective). Single-channel current recordings were made in the perijunctional region (within a distance of $20\text{--}30 \mu\text{m}$ of the endplate) of four fibres from two muscles. Patch pipettes had a tip diameter of $0.5\text{--}0.8 \mu\text{m}$. Pipette and extracellular solutions were as described in Fig. 1 legend, except that 80 mM glucose was added to give the same osmolality as Ringer's solution (290 mOsm). M.e.p.c.s were measured in sacro-coccygeal muscle bathed in Ringer's solution at $20 \pm 1^\circ \text{C}$, using a conventional two-microelectrode voltage-clamp circuit². The placement of the two microelectrodes within $50 \mu\text{m}$ of the endplate was verified after each measurement by staining the endplate acetylcholinesterase⁴² while the microelectrodes were left in place. M.e.p.c.s were low-pass filtered (2 kHz , -3 dB) and digitized at $100\text{-}\mu\text{s}$ intervals. M.e.p.c. decay time courses were fitted by eye with single exponentials. In each fibre, $20\text{--}60$ m.e.p.c.s were measured at each membrane potential. In all experiments, the distribution of the decay time constants had a single peak. The τ values shown in Fig. 3c represent means of such distributions.

ACh. Whole-cell current measurements showed that omission of the α -, β - or δ -subunit-specific mRNA virtually abolished the ACh response, whereas omission of either the γ - or the ϵ -subunit-specific mRNA did not appreciably affect the response as compared with oocytes injected with all five subunit-specific mRNAs. Figure 2a shows records of ACh-activated single-channel currents from outside-out patches isolated from oocytes injected with the α -, β -, γ - and δ -subunit-specific mRNAs (upper trace) or with the α -, β -, δ - and ϵ -subunit-specific mRNAs (lower trace). These oocytes, in contrast to those injected with all five subunit-specific mRNAs, each exhibited only a single class of AChR channels (AChR _{γ} or AChR _{ϵ}). The conductance and gating properties of the channels formed in a given oocyte depended on the mRNA combination with which it was injected. The distribution of current amplitudes for the AChR as well as the AChR _{ϵ} channel was characterized by a single peak at all membrane potentials. The $I-V$ relations for both channels in outside-out patches were linear in the potential range of -100 mV to $+50 \text{ mV}$ (Fig. 2b). The average conductance, obtained from both outside-out and cell-attached patches, was $40 \pm 1 \text{ pS}$ ($n = 16$) for the AChR _{γ} and $59 \pm 2 \text{ pS}$ ($n = 13$) for the AChR _{ϵ} channel (Table 1).

The conductance sequence of the two AChR channels for monovalent metal cations was $\text{K}^+ > \text{Cs}^+ > \text{Na}^+ > \text{Li}^+$, the respective channel slope conductances, measured in cell-attached patches at low extracellular Ca^{2+} (ref. 28), being $72 \pm 4 \text{ pS}$, $61 \pm 3 \text{ pS}$, $50 \pm 2 \text{ pS}$ and $21 \pm 2 \text{ pS}$ ($n = 3$) for the AChR _{γ}

channel and $110 \pm 4 \text{ pS}$, $90 \pm 5 \text{ pS}$, $60 \pm 4 \text{ pS}$ and $30 \pm 2 \text{ pS}$ ($n = 3$) for the AChR _{ϵ} channel. Thus, the conductance ratios determined for the four cations were nearly identical for the two AChR channels.

With respect to their conductances, the AChR _{γ} and AChR _{ϵ} channels resemble the AChR channels in fetal and adult rat muscle, respectively³. This resemblance is further supported by the conductance-activity curves for Na^+ of the AChR _{γ} and AChR _{ϵ} channels (Fig. 2c). These curves were fitted by hyperbolic relations, indicating that the AChR _{ϵ} channel has a larger limiting conductance (93 pS) than the AChR _{γ} channel (75 pS).

The AChR _{γ} and AChR _{ϵ} channels differed not only in their conductance but also in their gating properties. The average duration of elementary currents was estimated from the distribution of current durations²⁸ and from power density spectra^{1,31} of single-channel current records (Fig. 2d). In both channels, the average current duration decreased exponentially with more positive membrane potentials, but at all membrane potentials the average duration of AChR _{ϵ} channel currents was shorter by a factor of two to three than that of AChR _{γ} channel currents.

The results described above suggest that oocytes injected with all five subunit-specific mRNAs produce two types of AChR molecules which have different functional properties and are composed of the α -, β -, γ - and δ -subunits and of the α -, β -, δ - and ϵ -subunits, respectively. We also observed that oocytes injected with no more than the α -, β - and δ -subunit-specific mRNAs exhibited a strong response to ACh. Preliminary

analysis of the AChR channels formed in these oocytes indicates that their conductance and gating properties differ markedly from those of the AChR_γ and AChR_ε channels (our unpublished results).

AChR channels in muscle

To compare the two classes of AChR channels produced in *Xenopus* oocytes with the native bovine AChR channels, we examined the conductance and gating properties of ACh-activated channels in fetal and adult bovine muscle. The upper and lower traces in Fig. 3a show ACh-activated currents from outside-out patches isolated from fetal and adult muscle fibres, respectively. The distributions of current sizes in the two experiments were characterized by a single peak with different average values of 2.5 pA (fetal muscle) and 3.9 pA (adult muscle) at -60 mV membrane potential. The *I*-*V* relations were linear (Fig. 3b), and the average conductance was 40 ± 1 pS ($n = 8$) for the fetal AChR channel and 59 pS (mean of two patches) for the adult AChR channel (Table 1).

Table 1 Single-channel properties of two forms of bovine AChRs expressed in *Xenopus* oocytes and in muscle

AChR channels from	Conductance (pS)	Average duration	
		$\tau(-100)$ (ms)	<i>H</i> (mV)
Oocytes with AChR _γ	39 (7)	10.4 (7)	106
Oocytes with AChR _ε	59 (6)	5.3 (6)	83
Fetal muscle	40 (4)	11.0 (4)	108
Adult muscle	59 (2)	5.6 (6)	93

Regression analysis of *i*-*V* and τ -*V* measurements is shown. Numbers in parentheses refer to numbers of patches, except that two patches and four endplates were used for adult muscle. γ represents the slope of *i*-*V* relations measured on outside-out patches. $\tau(-100)$ represents the calculated duration of elementary currents at -100 mV membrane potential obtained from the τ -*V* relation plotted in semilogarithmic coordinates and fitted by the equation $\tau(V) = \tau(0) \exp(-V/H)$, where $\tau(0)$ is the τ value at 0 mV membrane potential. *H* represents the shift in membrane potential that causes an e-fold change in the average duration of elementary currents. The $\tau(0)$ values were 4.0 ms (AChR_γ) and 1.6 ms (AChR_ε) in Fig. 2d and 4.4 ms (fetal muscle) and 1.9 ms (adult muscle) in Fig. 3c. The correlation coefficients, r^2 , were 0.88 and 0.96 (Fig. 2d), and 0.92 and 0.98 (Fig. 3c). The averages of individual slope conductances, measured between the current reversal potential and -100 mV driving potential in both outside-out and cell-attached patches, were 40 ± 1 pS ($n = 16$) and 59 ± 2 pS ($n = 13$) for the AChR_γ and AChR_ε channels in oocytes, and 40 ± 1 pS ($n = 8$) and 59 pS (mean of 2 patches) for AChR channels in fetal and adult muscle, respectively. The averages of measured τ values at -100 mV membrane potential were 10.2 ± 1.4 ms ($n = 4$) and 5.2 ± 0.4 ms ($n = 3$) for the AChR_γ and AChR_ε channels in oocytes, and 12.5 ± 3.8 ms ($n = 4$) and 5.7 ± 0.4 ms ($n = 6$) for AChR channels in fetal and adult muscle, respectively.

The conductance sequence of the AChR channel in fetal muscle for monovalent metal cations was $K^+ > Cs^+ > Na^+ > Li^+$, with slope conductance values (measured in cell-attached patches at low extracellular Ca^{2+}) of 79 pS, 65 pS, 55 pS and 20 pS (one patch). For the AChR channel in adult muscle, the sequence was $K^+ > Cs^+ > Na^+$ with conductance values of 110 pS, 90 pS and 60 pS, respectively (one patch).

The average duration of ACh-activated elementary currents in fetal as well as adult muscle decreased exponentially with more positive membrane potentials (Fig. 3c, filled and open circles). The currents were shorter in adult muscle than in fetal muscle. For example, the average duration at -100 mV membrane potential was 12.5 ± 3.8 ms ($n = 4$) in fetal muscle and 6.0 ms (mean of two recordings) on the perijunctional region of adult muscle. The latter duration is considerably longer than that inferred for junctional channels in adult rat muscle from

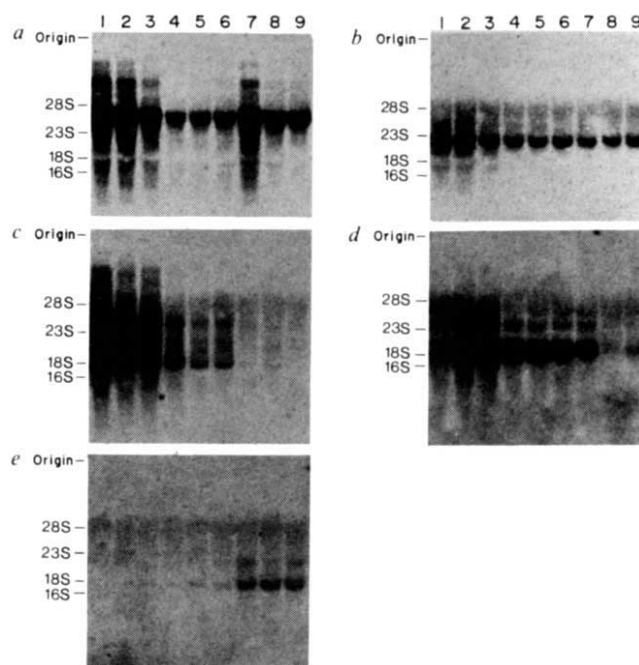


Fig. 4 Autoradiograms of blot hybridization analysis of total RNA from bovine diaphragms at various developmental stages using calf AChR α - (a), β - (b), γ - (c), δ - (d) and ϵ -subunit (e) cDNA probes. Bovine diaphragms obtained from a local abattoir were frozen quickly in liquid nitrogen and stored at -70°C . Fetal age was estimated by fetal body length⁴³. Total RNA was extracted by the guanidine thiocyanate method⁴⁴. Samples of total RNA (30 μg each) from diaphragms at various fetal (lanes 1-6) and postnatal (lanes 7-9) stages were denatured with 1 M glyoxal and 50% dimethyl sulphoxide⁴⁵, electrophoresed on a 1.5% agarose gel and transferred⁴⁶ to a Biotodyne nylon membrane (Pall); the ages of the animals were 3.4 months (lane 1), 4.4 months (lane 2), 5.3 months (lane 3), 5.7 months (lane 4), 7.1 months (lane 5) and 8.3 months (lane 6) of gestation and 5 h (lane 7), 25 days (lane 8) and ~ 2 yr (lane 9) after birth. The hybridization probes used were the 786-base-pair (bp) *Bgl*II/*Cl*AI fragment from clone pcACR α 18 (ref. 18; specific activity, 4.6×10^8 c.p.m. μg^{-1}) (a), the 1,791-bp *Ban*I fragment from pSPC β (ref. 28; 6.6×10^8 c.p.m. μg^{-1}) (b), the 980-bp *Hin*II/*Acc*I fragment from pcACR γ 5 (ref. 20; 8.4×10^8 c.p.m. μg^{-1}) (c), the 1,565-bp *Eco*RI/*Xba*I fragment from pSPC δ (ref. 28; 9.3×10^8 c.p.m. μg^{-1}) (d) and the 1,533-bp *Bam*HI/*Eco*RI fragment from pSPC ϵ (ref. 25; 8.1×10^8 c.p.m. μg^{-1}) (e); the probes were labelled by nick translation⁴⁷ with [α -³²P]dCTP. After prehybridization, the blots were incubated at 42°C for 21 h in a solution containing 50 mM sodium phosphate buffer (pH 6.5), 5 $\times$ SSC (SSC: 150 mM NaCl, 15 mM trisodium citrate), 50% formamide, 0.1% polyvinylpyrrolidone, 0.1% Ficoll 400 (Pharmacia), 0.1% bovine serum albumin, 250 $\mu\text{g ml}^{-1}$ sonicated salmon sperm DNA, 0.1% SDS and 10 ng ml^{-1} ³²P-labelled probe. The blots were washed three times, for 5 min each, in 2 $\times$ SSC containing 0.1% SDS at room temperature and twice, for 15 min each, in 0.1 $\times$ SSC containing 0.1% SDS at 50°C . Autoradiography was performed at -70°C for 70 h (a, b) or 140 h (c, d, e) with an intensifying screen. The size markers used were bovine and *Escherichia coli* ribosomal RNA. The estimated sizes of the α -, β -, γ -, δ - and ϵ -subunit mRNAs were $\sim 4,500$ (ref. 18), $\sim 3,200$ (ref. 19), $\sim 2,000$ (ref. 20), $\sim 2,200$ (ref. 21) and $\sim 1,900$ nucleotides, respectively. The $\sim 1,900$ -nucleotide ϵ -subunit mRNA was found in both diaphragm and leg muscle RNA. Our previous failure to detect this mRNA species²⁵ can be ascribed to the use of nitrocellulose filter, which yields weaker hybridization signals than Biotodyne nylon membrane; the previous observation of the $\sim 4,200$ -nucleotide RNA species²⁵ in poly(A)⁺ RNA from leg muscle from a fetal calf (4 months gestation) was not reproducible.

miniature endplate current (m.e.p.c.) decay times^{4-6,32}. We therefore measured m.e.p.c. decay time constant in adult bovine muscle (Fig. 3c, open squares). At -100 mV membrane potential, it was 5.6 ± 0.4 ms (four endplates), in good agreement with the average duration determined directly from single-channel current recordings. The voltage dependence of the m.e.p.c. decay time constant was similar to that of the duration of elementary currents (Fig. 3c). Thus, the estimates of the size and duration of elementary currents and their voltage dependence in bovine muscle indicate that the AChR channel in fetal muscle is similar

to the AChR_γ channel expressed in oocytes, whereas the AChR channel in adult muscle and the AChR_ε channel produced in oocytes share similar properties (Table 1).

AChR subunit mRNAs in muscle

We examined developmental changes in the contents of the five AChR subunit mRNAs in bovine muscle. Total RNA was extracted from diaphragms at various stages of fetal and postnatal development and subjected to blot hybridization analysis using the respective cDNA probes (Fig. 4). One major hybridizable RNA species was observed with the α - (Fig. 4a), β - (b) or ϵ -subunit (e) cDNA probe, whereas two (or three) major RNA species hybridized with the γ - (Fig. 4c) or δ -subunit (d) cDNA probe, especially at earlier fetal stages. The larger RNA species hybridizable with the γ - and δ -subunit cDNA probes, as well as the minor, larger RNA species hybridizable with the other cDNA probes, presumably represent incompletely spliced RNA^{20,21}. At all the developmental stages studied, significant amounts of the α - (Fig. 4a), β - (b) and δ -subunit (d) mRNAs were found, although the content of the δ -subunit mRNA was smaller at postnatal stages (especially 25 days and ~2 yr) than at fetal stages. In contrast, the contents of the γ - and ϵ -subunit mRNAs varied markedly during muscle development, showing reciprocal changes. The γ -subunit mRNA was found abundantly at earlier fetal stages (3–5 months gestation), but was hardly or not detectable after birth (Fig. 4c). Conversely, considerable amounts of the ϵ -subunit mRNA appeared only at postnatal stages, and it was not detectable at earlier fetal stages (3–4 months gestation) (Fig. 4e). At later fetal stages (7–8 months gestation), appreciable amounts of the γ - and ϵ -subunit mRNAs coexisted. Analogous experiments with poly(A)⁺ RNA from bovine leg muscles at different fetal and postnatal stages showed generally similar developmental changes in the contents of the five subunit mRNAs. These results support the notion that replacement of the γ -subunit by the ϵ -subunit in the AChR complex is responsible for the changes in the properties of the AChR that occur during muscle development^{4–6}. The reason for the low content of the δ -subunit mRNA observed at postnatal stages is not known. The possibility that replacement of the δ -subunit may also occur during muscle development cannot be excluded.

Conclusions

Two classes of AChR channels, defined by different conductances and gating properties, are formed in *Xenopus* oocytes injected with the five bovine AChR subunit-specific mRNAs. Only one of these two classes of AChR channels (AChR_γ) is produced by injection of the α -, β -, γ - and δ -subunit-specific mRNAs, whereas only the other class (AChR_ε) is produced by injection of the α -, β -, δ - and ϵ -subunit-specific mRNAs. These results suggest that AChR_γ and AChR_ε represent a complex of the α -, β -, γ - and δ -subunits and of the α -, β -, δ - and ϵ -subunits, respectively.

The two classes of channels, AChR_γ and AChR_ε, formed in oocytes have functional properties similar to those of the native bovine AChR channels present in fetal and adult muscle, respectively. This finding, in conjunction with the reciprocal changes in the contents of the γ - and ϵ -subunit mRNAs observed during muscle development, suggests that the AChR channel in fetal muscle is assembled from the α -, β -, γ - and δ -subunits, whereas the endplate channel in adult muscle is assembled from the α -, β -, δ - and ϵ -subunits. This conclusion is supported by the observation that the junctional AChR is slightly more acidic than the non-junctional AChR³³: the bovine ϵ -subunit contains more acidic residues and less basic residues than does the bovine γ -subunit²⁵.

The relative contents of the two classes of AChR channels, for example in rat muscle, depend on innervation of the muscle by spinal motoneurons^{4,34,35}. Before innervation, the fetal class of channels predominates; during synapse formation, the two AChR classes coexist in the muscle; at later stages of synapse formation, the fetal class is fully replaced by the adult class^{4–7,34}. Thus, the shift of the two AChR classes may occur concurrently with the reciprocal changes in the contents of the γ - and ϵ -subunit mRNAs. It seems plausible to postulate that the nerve terminal exerts control over the synthesis of the γ - and ϵ -subunits by switching the expression of their genes or by modulating some post-transcriptional events.

We thank Dr R. Eckert for reading the manuscript. This investigation was supported in part by research grants from the Ministry of Education, Science and Culture of Japan, the Mitsubishi Foundation and the Japanese Foundation of Metabolism and Diseases.

Received 17 March; accepted 8 April 1986.

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A second post-transcriptional *trans*-activator gene required for HTLV-III replication

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Evidence is provided for the existence of a seventh gene in the genome of human T-lymphotropic virus type III/lymphadenopathy-associated virus. The gene is necessary for replication and acts post-transcriptionally to relieve negative regulation of the messenger RNA for the virion capsid and envelope proteins. These observations suggest mechanisms for regulating both the latent and lytic phases of the virus life cycle.

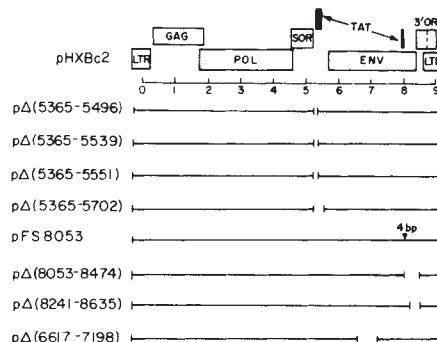
THE retrovirus human T-lymphotropic virus type III/lymphadenopathy-associated virus (HTLV-III/LAV) is the primary aetiological agent of AIDS (acquired immune deficiency syndrome) and associated disorders¹⁻⁶. The clinical consequences of HTLV-III infection range from prolonged asymptomatic states to severe depletion of T4⁺ helper lymphocytes, characteristic of frank AIDS, and profound degeneration of the central nervous system¹⁻⁷. The variable course of HTLV-III-related disease may reflect the complex life cycle of the virus, now thought to include both latent and lytic states in T4⁺ lymphocytes as well as a state of limited replication in monocytes and macrophages⁸.

To analyse viral factors affecting the HTLV-III life cycle, the effects of defined mutations of the viral genome on the replication and cytopathic potential of the virus have been studied. The HTLV-III genome, like that of other retroviruses, contains three open reading frames, *gag*, *env* and *pol*, which encode, respectively, the capsid proteins, the envelope proteins and

non-structural proteins necessary for replication⁹⁻¹⁷. In addition, the HTLV-III genome contains other open reading frames that encode at least three additional proteins not common to most retroviruses^{9-12,18,19}. Mutations in two of these open reading frames, the *sor* gene which encodes a protein of relative molecular mass (*M_r*) 23,000 (23K)^{20,21} and the 3' *orf* gene which encodes a 27K protein²², do not eliminate the ability of the virus to replicate in and kill T lymphocytes²⁰. The *trans*-activator (*tat*-III) gene encodes a 14K protein²³ that post-transcriptionally²⁴ stimulates HTLV-III long terminal repeat (LTR)-directed gene expression^{18,19,25} via an interaction with specific target sequences in the leader of viral messages²⁶. Mutations in the 5' portion of the first coding exon of the bipartite *tat*-III gene destroy the ability of the virus to efficiently synthesize structural proteins and to replicate²⁷. These mutations can be complemented in *trans* in cell lines that constitutively express the *tat*-III protein²⁷.

During these experiments, we found that an additional set of

Fig. 1 Structure and properties of the HTLV-III proviral mutants. The complete HTLV-III provirus on plasmid pHBc2 (ref. 28), together with the known genes, is shown at the upper left. The solid boxes represent the two coding exons of the *tat*-III gene. The vertical broken line in the 3' *orf* gene represents a stop codon present in the pHBc2 provirus²⁰. The scale beneath the viral genes represents kilobases. Numbers correspond to those in ref. 9, where the RNA cap site is designated +1. For proviral deletion mutants, the numbers in the plasmid name correspond to the endpoints of the corresponding deletion. All plasmids were made using restriction and modification enzymes, according to manufacturers' suggestions. The



Jurkat- <i>tat</i> -III				Raji			
Replication		Protein Production		RNA		Protein Production	
MIF	CPE	<i>gag</i>	<i>env</i>	<i>gag</i>	<i>env</i>	<i>tat</i>	TA
4	6	+	+	+	+	+	41.2
4	6	+	+	+	-	-	0.2
ND	ND	+	+	ND	-	-	0.2
> 30 days	-	-	-	ND	-	-	0.2
> 30 days	-	-	-	ND	-	-	0.2
> 30 days	-	-	-	+	-	-	39.3
> 30 days	-	-	-	+	-	-	35.6
7	9	+	+	ND	ND	ND	27.1
> 30 days	+	-	-	ND	ND	ND	43.5

4-bp insertion in the pFS8053 plasmid resulted from treating a *Bam*HI-digested provirus with the large fragment of DNA polymerase I in the presence of nucleotide triphosphates and re-ligating prior to transfection of *Escherichia coli*. Replicative potential of the proviruses was tested by transfection of CsCl-banded DNA into Jurkat (*tat*-III) cells²⁹ using the DEAE-dextran technique³¹. The values for membrane immunofluorescence (MIF) and cytopathic effect (CPE) represent the number of days after transfection that >95% HTLV-III-specific membrane immunofluorescence and >95% cytopathicity, respectively, were noted in a typical experiment. These values were assessed as described previously²⁰; >30 days indicates that no more than 2% HTLV-III-related membrane immunofluorescence or cytopathic effect was observed in the cultures, even up to 30 days after transfection. Reverse transcriptase assays³² of cell supernatants in these cases did not rise above background during the observation period. ND, not done. Production of viral RNA was assessed as described in Fig. 2 legend. Viral protein production was assessed by transfecting cells with 10 µg of plasmid DNA using the DEAE-dextran procedure³¹ and labelling with ³⁵S-cysteine at 48-72 h post-transfection. Labelled cell lysates were precipitated using antisera from patients (RV119 for Jurkat (*tat*-III) cells and 38-1 for Raji cells) and assessed for *gag*, *env* and *tat*-III protein production on SDS-polyacrylamide gels³³. + Indicates detectable *gag* (p55, p38, p24 and/or p17), *env* (gp160/120) or *tat*-III (p14) bands; - indicates no detectable level of these proteins above background. *Trans*-activating ability (TA) was assessed by co-transfecting 10 µg of the proviral plasmid with 10 µg of plasmid pU3R-III, containing HTLV-III LTR sequences from -457 to +80 5' to the chloramphenicol acetyltransferase gene (*cat*), into Raji cells^{34,35}; 48 h after transfection, cell lysates were assayed for *cat* enzyme activity as described. Values represent the percentage conversion of chloramphenicol to acetylated forms in 1 h using equivalent amounts of protein lysate in a typical experiment. No effect on *cat* activity directed by the pSV₂*cat* plasmid, containing the simian virus 40 (SV40) early region promoter 5' to *cat*³⁵, was observed with any of the mutant proviruses tested (data not shown).

mutations near the two coding exons of the *tat*-III gene dramatically attenuated the ability of the virus to express *gag* and *env* proteins. The observation that these mutations could not be complemented by the *tat*-III gene product alone led to the discovery of a second post-transcriptional control pathway governing HTLV-III expression. This pathway is controlled by a *trans*-acting product specified by a novel gene that partly overlaps the *tat*-III and *env* genes.

Replication of proviral mutants

To identify HTLV-III genomic regions important for viral replication, mutations were introduced into the pHXBc2 plasmid, which contains a complete HTLV-III provirus²⁸. On transfection of either primary lymphocytes or T4⁺ lymphocyte lines with this plasmid, infectious cytopathic virus is produced^{20,27,28}. The recipient cell used for these experiments is the Jurkat (*tat*-III) cell line, designed to express constitutively the *tat*-III protein²⁹. This cell line was chosen as it functionally complements deletions in the 5' coding exon of the *tat*-III gene and because it is highly sensitive to the cytopathic effects of HTLV-III²⁷. By 5 days after transfection with the pHXBc2 plasmid, Jurkat (*tat*-III) cells express HTLV-III proteins in addition to *tat*-III, exhibit cytopathic changes leading to demise of the culture, and produce extracellular virion particles detectable by electron microscopy or reverse transcriptase assays²⁷. A similar result with approximately the same time course was obtained after transfection with plasmid pΔ(5365–5496), which contains a deletion in the 5' portion of the *tat*-III first coding exon (Fig. 1). By contrast, no evidence of virus production was observed after transfection of the Jurkat (*tat*-III) cells with plasmid pΔ(5365–5551) or pΔ(5365–5702); these plasmids contain deletions in the *tat*-III gene which have the same 5' boundary as that of plasmid

pΔ(5365–5496), but extend farther in the 3' direction. These deletions do not extend into the *env* gene. Apparently a region between nucleotides 5,496 and 5,551 is required for HTLV-III replication, even in the presence of a functional *tat*-III product supplied in *trans*.

Mutations of the pHXBc2 plasmid in the region near the unique *Bam*HI site (position 8,053), including a 4-base pair (bp) insertion at this site, also inactivate viral replication in the Jurkat (*tat*-III) cells (Fig. 1). By contrast, a virus that contains a deletion in the 3' *orf* gene extending to within 188 nucleotides 3' to the *Bam*HI site retains the ability to replicate, albeit at a reduced rate compared with wild-type virus (plasmid pΔ(8241–8634) in Fig. 1). This observation suggests that a function necessary for viral replication is located very near the *Bam*HI site, 5' to nucleotide 8,241. Thus, mutations affecting two separate regions of the HTLV-III genome result in the inability of the provirus to replicate, even in the presence of the *tat*-III gene product.

RNA and protein synthesis of mutants

To investigate the nature of the mutations that could not be complemented by the *tat*-III product, the levels of virus-specific RNA and proteins synthesized by the proviral mutants were measured. The human B-lymphocyte cell line, Raji, was chosen for these experiments as previous experience demonstrated that significant levels of both virus-specific RNA and protein could be detected after transfection of this cell line with the HTLV-III proviral DNA²⁴.

For these experiments, RNAs and proteins were isolated 48 h after transfection of a single culture. Virus-specific RNA was measured using radiolabelled DNA complementary to HTLV-III RNA. The amount of globin RNA produced by the plasmid

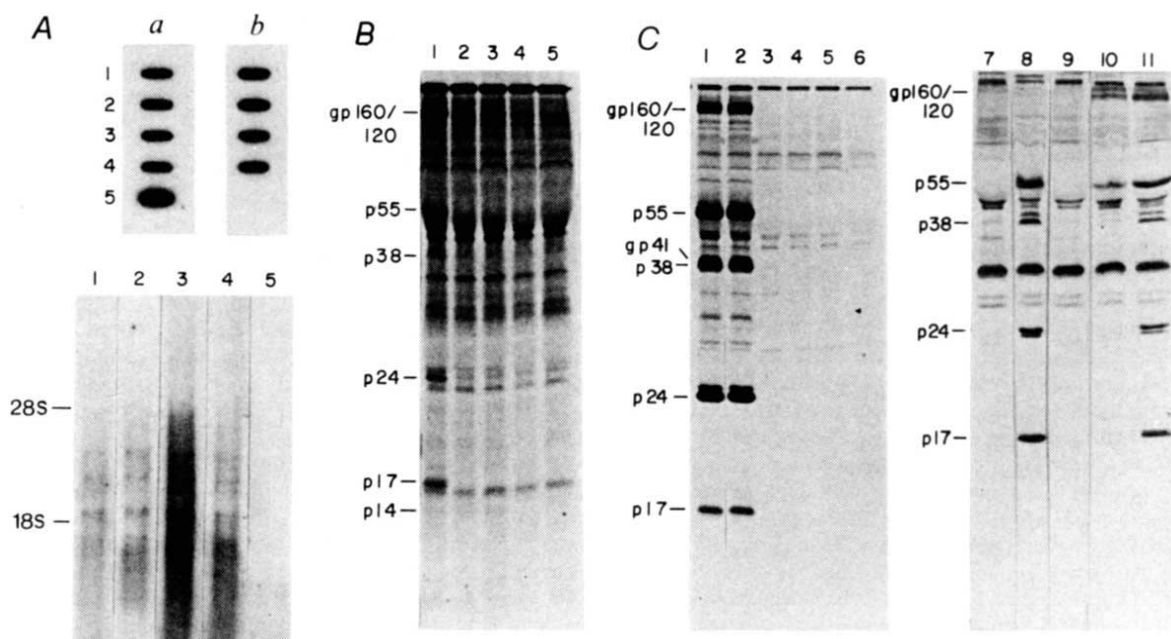


Fig. 2 RNA and protein production following transfection. Approximately 5×10^7 Raji cells were transfected with 10 μ g of test plasmid DNA and 10 μ g of pSV β -globin DNA using the DEAE-dextran technique³¹; 48 h after transfection, half of the cells were labelled with 35 S-cysteine and immunoprecipitated with patient antiserum 38-1, as described elsewhere³³. The other half of the cells was used for total RNA isolation using the guanidine thiocyanate–CsCl method³⁶. **A**, RNA (5 μ g) was slot-blotted onto duplicate nitrocellulose filters and 10 μ g was size-separated on formaldehyde gels and transferred to nitrocellulose³⁷. One slot blot was hybridized to a probe derived from the complete β -globin complementary DNA sequence (a); the other slot blot (b) and the Northern blot (lower figure) were hybridized to a probe made from a pooled collection of *Bgl*III internal proviral fragments derived from the plasmid pHXBc2. Filters were washed as described elsewhere³⁷ prior to autoradiography. **B**, Proteins immunoprecipitated from the Raji transfectants. Transfected test plasmids for the Northern blot, slot blots and protein gel were: lane 1, pHXBc2; 2, pΔ(8053–8474); 3, pFS8053; 4, pΔ(5365–5496); and 5, a plasmid, pCR1, containing an incomplete HTLV-I provirus. The control lanes (1 and 4) have been published as part of a separate study²⁴. **C**, Immunoprecipitates of Jurkat (*tat*-III) cells transfected with proviral plasmids using patient antiserum RV119, as described in Fig. 1 legend. Transfected plasmids were: lanes 1, 11, pHXBc2; lane 2, pΔ(5365–5496); 3, pΔ(5365–5702); 4, pΔ(8053–8474); 5, pFS8053; 6, 7, pIII β -globin, containing an HTLV-III LTR 5' to rabbit β -globin cDNA sequences²⁴; 8, pΔ(6617–7198); 9, pΔ(5365–5551); and 10, pΔ(5365–5539).

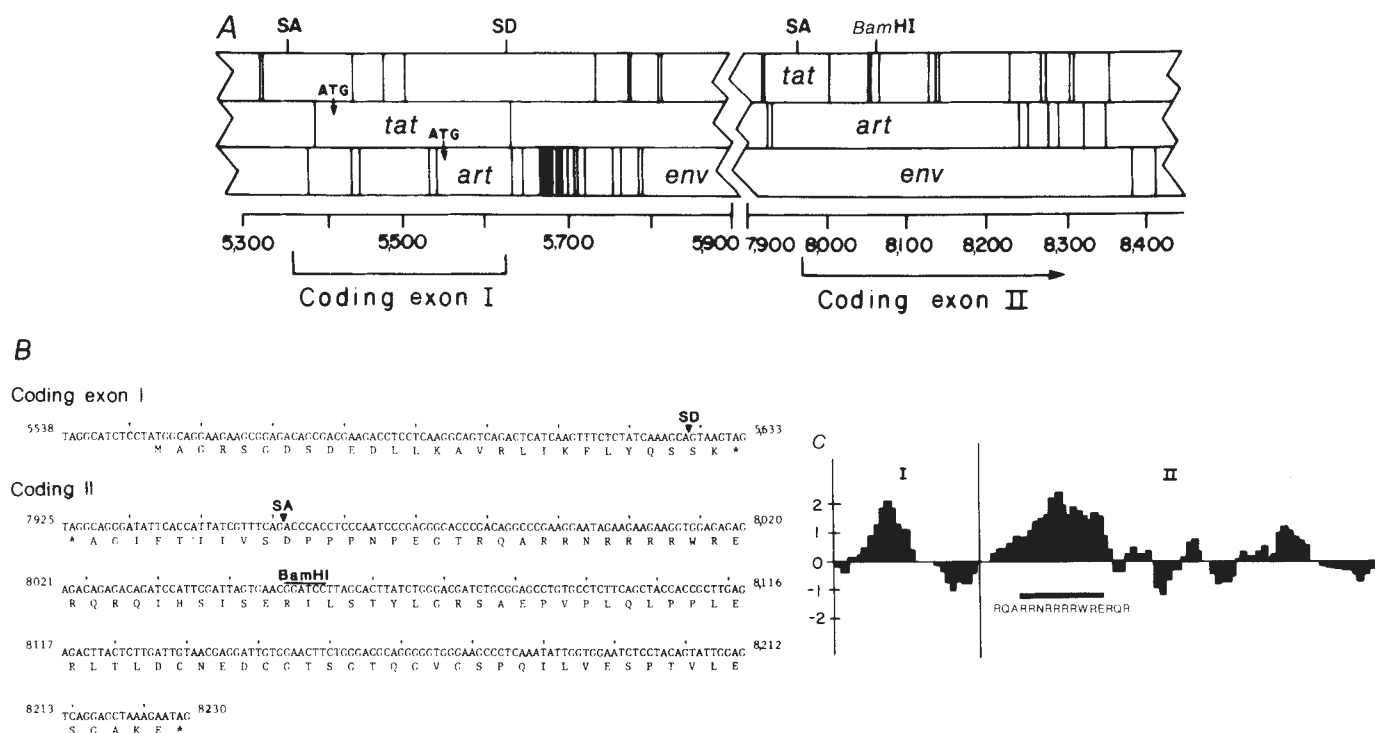


Fig. 3 Structure of the product of the alternative HTLV-III reading frame. The open reading frame in the HTLV-III genome, based on the sequence of Ratner *et al.*⁹, is shown in **A**. The vertical lines represent the positions of stop codons. The open reading frames for the *tat*-III, *env* and alternative reading frame (*art*) are indicated. The positions of known splice acceptor (SA) and donor (SD) sites¹² as well as the *Bam*HI site used for mutagenesis (position 8,053) are shown. The putative initiator methionine codons for the *tat*-III and alternative reading-frame products are indicated by arrows labelled ATG. The putative coding exons of the *tat*-III gene and the alternative reading frame are delineated beneath the figure. **B**, DNA sequence of the two open reading frames that constitute the alternative reading frame, with the positions of the probable splice donor (SD) and acceptor (SA) sequences¹² shown. The predicted amino-acid sequence of the potential product of the open reading frame is shown beneath the DNA sequence. If the given splice donor and acceptor sites are used, the amino-acid sequence encoded by the sequence near the splice site would be LYQSNPPPNP. **C**, Hydrophilic (positive)-hydrophobic (negative) profile of the predicted alternative reading-frame product, based on the program of Hopp and Woods³⁸. Protein domains specified by the first coding exon (I) are separated by a vertical line from those specified by the second coding exon (II). The amino-acid sequence of a strongly hydrophilic, basic domain is shown beneath the profile.

pSV2- β -globin³⁰, which was included in the transfection, was used as an internal standard for RNA synthesis in these experiments. Figure 2 shows that the amount, as well as the size distribution, of the virus-encoded RNA was the same for the pHXBc2 plasmid and for all the mutant HTLV-III proviruses tested, as judged by slot-blot and Northern blot analyses. Evidently the mutations that eliminate the ability of proviruses to produce infectious virus do not affect the synthesis of viral messenger RNA in the Raji cells.

The pattern of HTLV-III proteins produced in the transfected cells differed among the plasmids. Raji cells transfected with the pHXBc2 plasmid expressed HTLV-III-specific proteins of 160K and 120K (the *env* gene products), 55K, 38K, 24K and 17K (the *gag* gene products) and a faint band at 14K (the *tat*-III gene product) (Fig. 2B, lane 1). The pHXBc2 provirus used in these studies does not synthesize a complete 27K 3' *orf* product²⁰. Raji cells transfected with HTLV-III provirus containing *tat*-III gene deletions do not express detectable levels of viral protein, as reported previously²⁴ (Fig. 2B, lane 4). To our surprise, Raji cells transfected with p Δ (8053-8474) or pFS8053 failed to express HTLV-III *gag* or *env* gene products, but did synthesize the 14K *tat*-III product in amounts comparable to that seen after transfection with the pHXBc2 plasmid, as judged by radio-immune precipitation (Fig. 2B, lanes 2, 3). This observation suggested that mutations at or near position 8,053 attenuate the ability of the provirus to synthesize *gag* and *env* proteins, but do not affect synthesis of the *tat*-III protein.

To determine whether these mutant proviruses produced a functional *tat*-III gene product, the ability of the mutant pro-

viruses to activate the HTLV-III viral LTR in *trans* was measured. Figure 1 shows that the proviruses containing mutations in the 3' portion of the *env* gene are able to stimulate HTLV-III LTR-directed gene expression to approximately the same extent as is observed for the intact pHXBc2 provirus. We conclude that the mutations that affect *gag* and *env* gene expression do not significantly affect *tat*-III gene expression.

Non-complementation of the defect

Provirus containing deletions in *tat*-III cannot synthesize detectable levels of viral proteins on transient transfection into cell lines that do not express the *tat*-III product²⁴. Therefore, the Jurkat (*tat*-III) cell line was used to test the ability of mutant proviruses to synthesize virion proteins. Immunoprecipitation of Jurkat (*tat*-III) cells 48 h after transfection with the parental pHXBc2 plasmid revealed the presence of *gag* (p55, p38, p24 and p17) and *env* (gp160 and gp120) gene products (Fig. 2C, lane 1). HTLV-III *gag* and *env* proteins were also detected after transfection with the plasmids that contained deletions in the 5' portion of the *tat*-III first coding exon [p Δ (5365-5496) and p Δ (5365-5539) in Fig. 2C, lanes 2 and 10, respectively]. By contrast, no *gag* or *env* products were detected in Jurkat (*tat*-III) cells transfected with provirus containing deletions that extend at least 12 nucleotides farther 3' [p Δ (5365-5551) and p Δ (5365-5702) in Fig. 2C, lanes 9 and 3, respectively]. Similarly, none of the proviruses mutated near the *Bam*HI site (position 8,053) that failed to make viral *gag* and *env* proteins in Raji cells yielded detectable levels of these proteins in Jurkat (*tat*-III) cells (Fig. 2C, lanes 4, 5). The provirus with a deletion in the

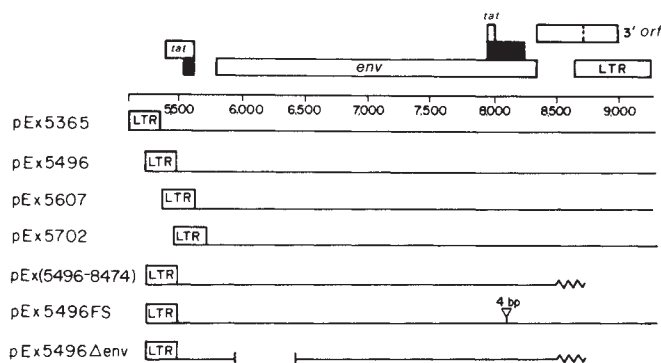


Fig. 4 Structure of plasmids containing the alternative reading frame. The structure of the 3' half of the HTLV-III genome, based on the sequence of Ratner *et al.*⁹, is shown together with the positions of the *env* gene, LTR, 3' *orf* gene, two *tat*-III coding exons and the putative alternative reading-frame coding exons (here depicted as solid boxes). The position of a stop codon in the 3' *orf* gene of the parental pXBC2 plasmid^{20,28} used in these studies is denoted by a vertical broken line. In all the plasmids shown, HTLV-III LTR sequences from -167 to +80 are positioned at the nucleotide denoted in the plasmid name. The zig-zag lines represent signals for polyadenylation and splicing derived from the SV40 early region³⁰. The 4-bp insertion in plasmid pEx5496FS was constructed as described for plasmid pFS8053 in Fig. 1 legend. pEx5496Δenv was constructed by digesting pEx(5496-8474) with *Stu*I, ligating to 8-bp *Kpn*I linkers, digesting to completion with *Kpn*I, and ligating prior to *E. coli* transfection. All plasmids were constructed using DNA modification and restriction enzymes, by standard procedures.

3' *orf* gene on plasmid pΔ(8241-8635) synthesized both *gag* and *env* proteins after transfection into Jurkat (*tat*-III) cells (Fig. 1). These experiments suggest that a region having a 5' boundary between nucleotides 5,539 and 5,551 and a 3' boundary between 8,053 and 8,241 is necessary for efficient expression of viral *gag* and *env* proteins, even in the presence of a functional *tat*-III product.

A seventh open reading frame

The similarity in the phenotype of mutations located both 5' to the *env* gene and within the 3' portion of the *env* gene suggested that these mutations might affect a single gene product. That mutation of the *env* gene product is not alone responsible for the effects observed is demonstrated by the ability of the proviruses on plasmids pΔ(6617-7198) and pΔ(8241-8635) to produce *gag* proteins on transfection into Jurkat (*tat*-III) cells (Figs 1 and 2C, lane 8). Plasmid pΔ(6617-7198) contains a deletion-frameshift mutation near the middle of the *env* gene, while plasmid pΔ(8241-8635) contains a deletion involving the 3' 132 nucleotides of the *env* gene.

We note the existence within the HTLV-III genome of a sequence that could encode a protein and that would be affected by both sets of mutations affecting *gag* and *env* expression. The postulated coding exons of this gene would use alternative reading frames of the first and second coding exons of the *tat*-III gene (Fig. 3). Beginning with a methionine codon at position 5,550, the first coding exon of the putative gene extends to a known splice donor site at position 5,625 (ref. 12). The corresponding splice acceptor, located at nucleotide 7,956, precedes an in-frame open reading frame ending in a stop codon at position 8,227. The splicing events needed to produce this alternative reading-frame product are the same as those used for the *tat*-III gene and are known to occur in the mRNA of HTLV-III-infected cells^{12,18,19}.

The potential product of the alternative reading frame is 116 amino acids long, and contains stretches of highly basic hydrophilic residues similar to those found in the *tat*-III gene product and in nucleic acid-binding proteins^{18,19}. Note that the vectors

used for the construction of the Jurkat (*tat*-III) and Raji (*tat*-III) cells are truncated at the *Bam*HI site (position 8,053) and are unable to synthesize the alternative reading-frame product.

A novel *trans*-activator

The hypothesis that a protein encoded by the alternative reading frame described above is necessary, in addition to the *tat*-III product, for efficient HTLV-III *gag* and *env* protein synthesis, would explain the phenotype of the proviral mutants. This hypothesis predicts that plasmids designed to express the alternative reading-frame product should complement the defect of mutants with respect to the synthesis of the *gag* and *env* gene products. To test this prediction, HTLV-III LTR sequences from position -167 to +80 were placed at various positions with respect to the putative initiation codon for the alternative reading frame (Fig. 4). Following co-transfection of these plasmids with plasmids containing mutant proviruses into Jurkat (*tat*-III) cells, viral protein expression was assessed by immunoprecipitation of labelled cell lysates using antiserum from an AIDS patient.

Figure 5 illustrates that placing the HTLV-III LTR 54 nucleotides 5' to the ATG codon of the alternative reading frame (and downstream of the *tat*-III initiation codon) results in a plasmid (pEx5496) that provides in *trans* a function required for *gag* gene expression by the pFS8053 plasmid (Fig. 5B, lane 3 and C, lane 4). The *env*-encoded gp160/120 proteins are produced by the pEx5496 plasmid itself on transfection into Jurkat (*tat*-III) cells (Fig. 5A, lane 3). The ability of the pEx5496 plasmid to complement the pFS8053 mutation and to synthesize envelope proteins is eliminated by a frameshift mutation within the alternative reading frame (plasmid pEx5496FS; Fig. 5C, lane 7 and D, lane 3). Plasmids in which the HTLV-III LTR is located 3' to the ATG codon of the alternative reading frame do not complement the pFS8053 mutation and do not synthesize *env* gene products (plasmids pEx5607 and pEx5702 in Fig. 5A, lanes 4, 5 and B, lanes 4, 5). By contrast, deletions within the *env* or 3' *orf* gene of plasmid pEx5496 do not eliminate the ability of the plasmid pFS8053 to activate *gag* gene expression (see plasmids pEx5496Δenv and pEx(5496-8474) in Fig. 5C, lanes 5, 6 and D, lane 5). HTLV-III *gag* gene expression directed by the plasmid pΔ(8053-8474) can also be activated by pEx5496 (data not shown). We conclude that plasmids capable of producing an alternative reading-frame product can activate, in *trans*, *gag* gene expression by proviruses containing mutations in the alternative reading-frame sequence.

We also note that *gag* gene expression by the plasmid pFS8053 can be activated in Jurkat (*tat*-III) cells by a plasmid that contains the HTLV-III sequences located 5' to the initiation codon of the *tat*-III gene. The plasmid pEx5365 produces a functional *tat*-III activity as well as providing in *trans* the functions required for *gag* gene expression by plasmid pFS8053 (Fig. 5B, lane 2). However, the extent of *gag* gene expression observed in this experiment was less than that observed in the experiments in which pEx5496 was used. pEx5365 does not produce detectable levels of the *env* gene product (Fig. 5A, lane 2). One possible explanation for the low level of expression of the complementing activity and of *env* gene products by plasmid pEx5365 is the presence of a strong *tat*-III initiation codon 5' to both genes.

Activation of *env* gene expression

A plasmid designed to express the alternative reading frame but not the *env* gene was tested for the ability to allow *env* gene production by HTLV-III proviruses containing mutations in the alternative reading-frame sequences. The plasmid pEx5496Δenv, which contains a deletion-frameshift mutation in the portion of the *env* gene encoding the exterior glycoprotein gp120, did not yield detectable levels of the *env* protein on transfection into Jurkat (*tat*-III) cells (Fig. 5D, lane 2). Co-transfection of this plasmid into Jurkat (*tat*-III) cells with pΔ(5365-5551) allowed the latter plasmid to produce both *gag*

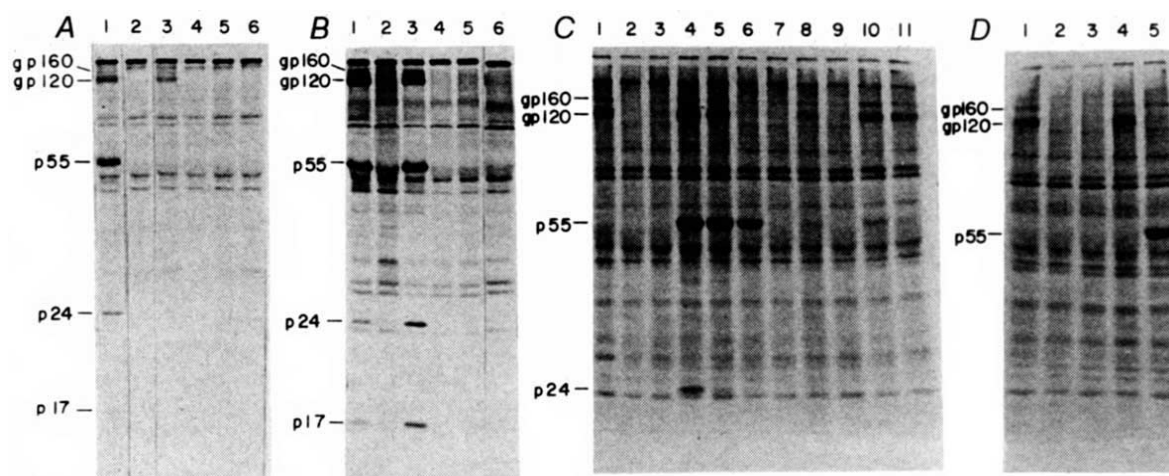


Fig. 5 Complementation of mutations in HTLV-III proviruses by plasmids designed to express the alternative reading-frame product. Jurkat (*tat*-III) cells were transfected by the DEAE-dextran procedure³¹ using 10 µg of the proviral mutant to be complemented and 10 µg of the plasmid to be tested for ability to complement the mutation. Forty-eight hours after transfection, cells were labelled with ³⁵S-cysteine and cell lysates were immunoprecipitated using an AIDS patient serum, RV119 (ref. 33). The positions of the gp160 and gp120 *env* proteins and of the p55, p24 and p17 *gag* gene products are indicated. Transfected plasmids in **A** were pΔ(5365–5496) (lane 1), pEx5365 (lane 2), pEx5496 (lane 3), pEx5607 (lane 4), pEx5702 (lane 5) and pIIIβ-globin (lane 6); those in **B** were pΔ(5365–5496) (lane 1), pFS8053 plus pEx5365 (lane 2), pFS8053 plus pEx5496 (lane 3), pFS8053 plus pEx5607 (lane 4), pFS8053 plus pEx5702 (lane 5), and pFS8053 alone (lane 6); and those in **C** were pEx(5496–8474) (lane 1), pEx5496Δenv (lane 2), pEx5496FS (lane 3), pFS8053 plus pEx5496 (lane 4), pFS8053 plus pEx(5496–8474) (lane 5), pFS8053 plus pEx5496Δenv (lane 6), pFS8053 plus pEx5496FS (lane 7), pΔ(5365–5551) plus pEx5496Δenv (lane 8), pΔ(5365–5551) plus pEx5496FS (lane 9), pΔ(5365–5539) plus pEx5496Δenv (lane 10), and pΔ(5365–5539) plus pEx5496FS (lane 11). Transfected plasmids in **D** were pEx(5496–8474) (lane 1), pEx5496Δenv (lane 2), pEx5496FS (lane 3), pEx5496Δenv plus pEx5496FS (lane 4), and pEx5496Δenv plus pFS8053 (lane 5). The predicted *env* gene product synthesized by the pEx5496FS plasmid is 47 amino acids shorter than the wild-type HTLV-III envelope, because of the frameshift mutation.

and *env* proteins (Fig. 5C, lane 8). No viral proteins could be detected in Jurkat (*tat*-III) cells transfected either with pΔ(5365–5551) alone (Fig. 2C, lane 9) or with plasmids pΔ(5365–5551) and pEx5496FS (Fig. 5C, lane 9). pEx5496Δenv also activated the synthesis of the gp160/120 *env* products by plasmids pEx5496FS (Fig. 5D, lane 4) and pEx5702 (data not shown). No *env* gene products were detected on transfection of Jurkat (*tat*-III) cells with either pEx5496FS alone (Fig. 5D, lane 3) or pEx5702 alone (Fig. 5A, lane 5). This result suggests that the inability of these plasmids to synthesize envelope proteins is the result of disruption of the alternative reading-frame sequences rather than of *cis*-acting sequences required to produce envelope proteins.

We conclude that a product encoded by the alternative reading frame can activate, in *trans*, *env* gene expression directed by proviruses mutated in the alternative reading frame.

Discussion

Here we have presented evidence for a novel *trans*-acting function required for efficient synthesis of HTLV-III *gag* and *env*, but not *tat*-III, proteins. In the absence of this second *trans*-acting factor, HTLV-III *gag* and *env* protein synthesis is markedly attenuated, without an apparent change in viral mRNA production. This finding, together with the observation that *tat*-III expression is not dependent on the *trans*-acting function, indicates that the factor exerts its effect at a post-transcriptional level.

The region of the genome required for this second *trans*-acting function is distinct from, but partly overlaps, the *tat*-III and *env* genes. This bipartite region contains an open reading frame that, on splicing, could synthesize a 116-amino-acid, highly basic protein similar to the *tat*-III product. This open reading frame is conserved in all HTLV-III variants sequenced^{9–12}.

The novel *trans*-acting function defined here activates HTLV-III *gag* and *env* protein expression; this might occur via a positive stimulation of post-transcriptional events affecting utilization of *gag* and *env* mRNA. Alternatively, the second *trans*-acting factor might derepress *cis*-acting negative-regulatory sequences present on viral messages encoding *gag* and *env*

products. We favour the latter explanation based on our observations regarding the expression of heterologous genes under the control of the HTLV-III LTR.

We have demonstrated previously that the *tat*-III product alone is sufficient to stimulate HTLV-III LTR sequences (–167 to +80) to direct high-level synthesis of bacterial chloramphenicol acetyltransferase or murine dihydrofolate reductase^{24,25}. By contrast, plasmids that contain the same HTLV-III LTR sequences located 5' to the HTLV-III *env* gene (pEx5486FS or pEx5702) yield no detectable envelope protein in the absence of the alternative reading-frame product, even in the presence of an active *tat*-III gene product. Only after the alternative reading-frame product is supplied in *trans* do the levels of envelope production by these plasmids approximate those of heterologous genes under the control of the HTLV-III LTR in Jurkat (*tat*-III) cells. These observations imply that in the plasmids pEx5496FS and pEx5702 sequences 3' to +80 inhibit *env* gene expression, and that such inhibition can be relieved by the *trans*-acting function. That the second *trans*-activator is required for *gag* gene expression suggests that *gag* mRNA may contain negative-regulatory sequences in addition to those found in *env* mRNA. We suggest the name *art* (anti-repression *trans*-activator) for the gene that encodes the second *trans*-activator, consistent with the proposed role of this gene in negating the effects of *cis*-acting negative-regulatory sequences present on viral mRNA encoding the HTLV-III structural proteins.

Detectable levels of the *tat*-III gene product but not of the *gag* and *env* gene products are synthesized by proviral mutants defective for the *art* function; therefore whatever inhibits the expression of the *gag* and *env* gene products does not affect *tat*-III gene expression. Sequences encompassing the entire *gag* gene and most of the *env* gene are removed by splicing from the *tat*-III mRNA^{12,18,19}. Removal of *art*-responsive *cis*-acting negative-regulatory sequences located in the regions spliced out of *tat*-III messages would explain the independence of *tat*-III expression from the requirement for the *art* product. The *art* product itself should be independent of such negative-regulatory sequences as it should be synthesized from messages that also lack these *gag* and *env* sequences.

The expression of HTLV-III structural genes is governed post-transcriptionally by the *tat*-III product, which acts as a positive regulatory factor^{18,19,24}, and by the *art* product, which apparently counteracts *cis*-acting negative-regulatory sequences located in or near the *gag* and *env* genes. Two possible roles for this complex regulatory scheme can be considered. HTLV-III is reported to establish a latent state of infection in T cells that are not activated⁸. Lack of *tat*-III or *art* function would lead to a state of infection characterized by accumulation of viral RNA without synthesis of virus structural proteins. Rapid release from such a latent state could be achieved in the absence of new RNA synthesis if the *tat*-III or *art* function were reconstituted. A dependence of *tat*-III and *art* activity on the state of cell differentiation could explain the relationship between HTLV-III latency and T-cell activation.

Alternatively, post-transcriptional regulators may play a part in the lytic cycle of the virus. An early stage of infection characterized by accumulation of viral RNA but not of virion proteins might precede a late phase in which viral proteins toxic to T4 cells are produced. The switch from an early to a late stage of infection would reflect activation of either one or both of the *tat*-III and *art* gene functions. Such an early-late switch would permit accumulation of the mRNAs for toxic virion components before such components themselves are produced. There is some evidence that modulated *tat*-III activity may result in increased production of infectious virus. The yield of virus particles on lytic infection of the Jurkat cell line that constitutively produces high levels of the *tat*-III gene is much lower than that observed on infection of the Jurkat cell line itself, despite the observation

that the cytopathic effect of infection is accelerated in the Jurkat (*tat*-III) cells²⁷. Premature cell death attributed to high constitutive levels of the *tat*-III gene might explain the decreased virus titres. Temporal regulation of virus gene expression is important in the life cycle of other lytic viruses. The postulated roles of the *tat*-III and *art* genes in the latent and lytic cycles of HTLV-III infection are not necessarily exclusive.

A flexible, multi-tiered regulatory pathway linked to host cell differentiation and proliferation could account for much of the variability observed in victims of AIDS⁶. Similar pathways involving both positive and negative elements may also apply to post-transcriptional regulation of gene expression in the course of normal cellular development. Note also that both the *tat*-III and *art* products provide attractive targets for antiviral agents, as both operate at a post-transcriptional level and are required for viral replication^{24,27}.

We thank Drs George Shaw, Beatrice Hahn, Flossie Wong-Staal and Robert Gallo for the gift of the pHXBc2 provirus; Mary Fran McLane, Myron Essex, David Ho and Martin Hirsch for gifts of antisera; Kathryn Campbell, Judith Lippke, Renee Zaya and Dennis Perkins for technical assistance; Patricia Baldacci for help with some of the experiments; Roberto Patarca and Daniel Celander for helpful discussions; and Tanya Parks for manuscript preparation. W.H. is supported by a State of Massachusetts contract and by NIH grant CA21082. J.S. is a Special Fellow of the Leukemia Society of America and is also the recipient of NIH grants CA40658 and CA42597. C.R. is a recipient of NIH postdoctoral fellowship CA07580. E.T. was supported by a NIH Virology Training Grant.

Received 20 February; accepted 2 April 1986.

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LETTERS TO NATURE

Towards the geometrization of physics

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The concept of symmetry breaking may be expressed mathematically by the reduction of a structure group, for example, choosing a point on a sphere reduces its symmetry group from the group of all rotations to the subgroup of rotations fixing that point. A systematic analysis of Penrose's twistor equation^{1,2} has revealed that twistor theory embodies a truth of nature: that interactive gravitational field theories in space-time correspond to such a symmetry breakdown—a breakdown that occurs, not in space-time, but in twistor space—the space of null geodesics of space-time. In particular, twistor theory lies at the heart of supergravity.

The Penrose transform^{3,4} provides the necessary algorithm linking space-time and twistor concepts. Applied to bundles, it

encodes the information of bundles with connection, on space-time, in bundles without connection, on twistor space. This is possible because null geodesics, which are local in twistor space (points), are extended in space-time. The information stored in parallel propagation along geodesics is completely used in building the geometry of the twistor bundle.

Remarkably, one can construct a bundle with connection, in space-time, the bundle of local twistors, such that: (1) the reduction of its structure group, combined with, (2) the simultaneous reduction of the connection, give the relevant field equations. Applying the Penrose transform, the field equations are expressed by a reduction of the structure group, in twistor space: the breakdown of symmetry.

For Dirac spinor fields ω and π , the twistor equation is:

$$\nabla_a \omega - \gamma_a \pi = 0 \quad (1)$$

Here ∇_a is a spin connection; the indices are abstract; γ_a obeys the Clifford algebra: $\gamma_a \gamma_b = g_{ab}$ (g_{ab} is the metric).

Equation (1) is invariant under the replacements:

$$\nabla_a \rightarrow \nabla'_a \equiv \nabla_a + \gamma_a \tau, \quad \omega \rightarrow \omega' \equiv \omega, \quad \pi \rightarrow \pi' \equiv \pi + \tau \omega$$

This structure creates a short exact sequence of bundles:

$$0 \rightarrow \Pi \xrightarrow{i} T \xrightarrow{j} \Omega \rightarrow 0 \quad (2)$$

where Π , Ω and T are, in turn, the bundles of spinors π , ω and of local twistors (ω^B); also $i(\pi) \equiv \binom{0}{\pi}$ and $j(\omega) \equiv \omega$.

It is fundamental that the integrability conditions of equation (1) allow one, for space-time dimension $m > 2$, to express the derivative $\nabla_a \pi$ linearly in terms of ω and π : $\nabla_a \pi = T_a \pi + R_a \omega$ (T_a is determined by $\nabla_a \gamma_b$ and by the torsion of ∇_a); in terms of the curvature R_{ab} of ∇_a , R_a is given by:

$$R_{ab} \equiv C_{ab} - \gamma_{[a} R_{b]}, \quad \gamma^a C_{ab} = 0 \quad (3)$$

Accordingly, one obtains a canonical connection D_a on T :

$$D_a \begin{pmatrix} \omega \\ \pi \end{pmatrix} \equiv \begin{pmatrix} \nabla_a \omega - \gamma_a \pi \\ \nabla_a \pi - T_a \pi - R_a \omega \end{pmatrix} \quad (4)$$

giving a one to one correspondence between solutions of the twistor equation and parallelly propagated local twistors.

Next two maps $t: \Omega \rightarrow T$, $p: T \rightarrow \Pi$ split the sequence (2) if they obey $jt = \text{identity}$, $pi = \text{identity}$, $pt = \text{zero}$. The connection D_a also splits, inducing connections on Ω and Π , provided the fundamental splitting equation holds:

$$pD_a t = 0 \quad (5)$$

One says that D_a splits directly if the induced connection on Ω is ∇_a . This amounts to the requirement, in equation (4), the twistors of the form $\binom{\omega}{0}$ are preserved by D_a . From equation (4) this is just the equation $R_a = 0$. Let ${}^L\nabla_a$ be the Levi-Civita connection. Then for ∇_a given successively by:

- (i) for $m > 2$, $\nabla_a \equiv {}^L\nabla_a$,
- (ii) for $m = 4$, $\nabla_a \equiv {}^L\nabla_a - (F_a{}^b \gamma_b - \frac{1}{4} \gamma_a F_{bc} \gamma^b \gamma^c) \gamma_5$,
- (iii) for $m = 11$, $\nabla_a \equiv {}^L\nabla_a - F_a{}^{bcd} \gamma_b \gamma_c \gamma_d + \frac{1}{12} \gamma_a F^{bcde} \gamma_b \gamma_c \gamma_d \gamma_e$,

(where F_{ab} and F_{abcd} are completely skew), then the condition of direct splitting of the local twistor connection gives: (i) the Einstein vacuum equations⁵; (ii) the full Einstein-Maxwell equations; (iii) the full equations of classical 11-dimensional supergravity⁶.

Example (iii) strongly suggests that there should be a super-symmetric generalization of the above formalism, since after all the construction of the equations of (iii) was first made using supersymmetry. To see how to achieve this, consider the original twistor equation of Penrose, the equation for a Weyl spinor ω^A :

$$\nabla_{A'} ({}^A \omega^B) = 0 \quad (6)$$

In classical supergravity the derivative $\nabla_{A'}$ factorizes⁷:

$$\nabla_{A'} = \bar{\nabla}_A \wedge \nabla_{A'} \equiv \bar{\nabla}_A \nabla_{A'} + \nabla_{A'} \bar{\nabla}_A \quad (7)$$

(Here $\wedge$ is the graded commutator: an anti-commutation for a pair of operators with an odd number of spinor indices; otherwise it is a commutation). Equation (6) now becomes:

$$\nabla_{A'} (\bar{\nabla}^A \omega^B) + \bar{\nabla}^A (\nabla_{A'} \omega^B) = 0 \quad (8)$$

This immediately suggests that the correct generalization of equation (6) should be the graded twistor equations:

$$\bar{\nabla}^A (\omega^B) = 0 \quad (9)$$

$$\nabla_{A'} \omega^B = 0 \quad (10)$$

Remarkably, this first guess works. In Table 1, the algebra generated by $\bar{\nabla}_A$ and $\nabla_{A'}$ is given, for the theory of minimal classical supergravity⁷. $\bar{\nabla}_{AB}$ and $\nabla_{A'B'}$ generate the Lorentz algebra. Using Table 1, one now analyses the integrability of equations (9) and (10). First rewrite equation (9):

$$\bar{\nabla}_A \omega^B = \delta_A{}^B \pi, \quad (11)$$

for some π . Define the spinor $\pi_{A'}$ in terms of π : $\pi_{A'} \equiv \nabla_{A'} \pi$. Then it is routine to establish that all derivatives of the *graded*

Table 1 The minimal classical supergravity algebra

$\bar{\nabla}_{AB} \wedge \bar{\nabla}_{CD} = 4\epsilon_{(A(C} \bar{\nabla}_{D)B)}$	$\bar{\nabla}_{AB} \wedge \nabla_{C'D'} = 0$	$\bar{\nabla}_{AB} = \bar{\nabla}_{BA}$
$\bar{\nabla}_{AB} \wedge \nabla_{C'C} = -2\epsilon_{C(A} \bar{\nabla}_{B)C'}$	$\bar{\nabla}_{AB} \wedge \bar{\nabla}_C = -2\epsilon_{C(A} \bar{\nabla}_{B)}$	$\bar{\nabla}_{AB} \wedge \nabla_{C'} = 0$
$\bar{\nabla}_A \wedge \bar{\nabla}_B = 2Q \bar{\nabla}_{AB}$	$\bar{\nabla}_A \wedge \nabla_{A'} = \nabla_{AA'}$	
$\bar{\nabla}_A \wedge \nabla_{BB'} = \epsilon_{AB} Q_{B'} + R_{ABB'}$		
$\nabla_{AA'} \wedge \nabla_{BB'} = \epsilon_{AB} Q_{A'B'} + \epsilon_{A'B'} \bar{Q}_{AB} + \epsilon_{AB} R_{A'B'} + \epsilon_{A'B'} \bar{R}_{AB}$		
$Q_{B'} \equiv 2Q \nabla_{B'} + Q_{B'}^B \bar{\nabla}_B$		
$Q_{A'B'} \equiv (\nabla_{(A'} Q_{B')}) \bar{\nabla}_{B'} + 2Q_{A'B'}^C \nabla_C + 2(\nabla_{(A'} Q) \nabla_{B')} + Q_{A'B'}^B \nabla_{B'}$		
$R_{ABB'} \equiv -(\nabla_{B'} Q) \bar{\nabla}_B + \epsilon_{AB} (-\frac{1}{2} (\bar{\nabla}^C Q_{B'}) \bar{\nabla}_{CD} + Q_{B'}^{C'D'} \nabla_{C'D'})$		
$R_{A'B'} \equiv -\frac{1}{2} (\nabla_{(A'} \bar{\nabla}^C Q_{B')}) \bar{\nabla}_{CD} + (\nabla_{(A'} Q_{B')}^C) \nabla_{C'D'}$		
$-\frac{1}{2} (\bar{\nabla}_C \bar{\nabla}^C \bar{Q} - 8Q \bar{Q}) \nabla_{A'B'}$		
$Q_{AA'} + \bar{Q}_{AA'} = 0$	$Q_{A'B'C'} = Q_{(A'B'C')}$	$\bar{\nabla}_A Q_{A'B'C'} = 0$
$\bar{\nabla}_A Q = 0$	$\bar{\nabla}_A Q^{ABC} = \frac{1}{2} \bar{\nabla}^B (Q^C A')$	$\bar{\nabla}_A Q^A + 2\nabla_{A'} Q = 0$

twistor ($\omega^B, \pi_{B'}, \pi$) may be computed linearly in terms of ($\omega^B, \pi_{B'}, \pi$). This gives immediately the definition of the *graded twistor connection* on GT , the bundle of graded twistors. The definition is presented in Table 2.

The defining property of the connection is that there be a one-to-one correspondence between solutions of the graded twistor equations and covariantly constant graded twistors. There is now a split exact sequence of graded bundles:

$$0 \rightarrow G\Pi \xrightarrow{i} GT \xrightarrow{j} G\Omega \rightarrow 0 \quad (12)$$

generalizing equation (2);

$$\text{here } i \left(\begin{pmatrix} \pi_{B'} \\ \pi \end{pmatrix} \right) \equiv \begin{pmatrix} 0 \\ \pi_{B'} \\ \pi \end{pmatrix}, \quad j \left(\begin{pmatrix} \omega^B \\ \pi_{B'} \\ \pi \end{pmatrix} \right) \equiv \omega^B$$

The graded analogue of the direct splitting is the condition that the graded twistors ($\omega^B, 0, 0$) are preserved by the graded connection.

By inspection of Table 2, one immediately finds the equations:

$$Q = 0, \quad \bar{\nabla}_{(A} Q_{B)B'} = 0 \quad (13)$$

Given equation (13) the induced connection on $G\Pi$ itself splits, preserving spinors of the form $\binom{0}{\pi}$, if and only if the slightly stronger system of equations holds:

$$Q = 0, \quad Q_{BB'} = 0. \quad (14)$$

Equation (13) gives the standard curvature field equations of classical supergravity. Equation (14), the second level of splitting, gives the standard torsion field equations⁷.

Finally, if equation (10) is generalized to the equation:

$$\nabla_{A'} \omega^B = \bar{F}^B \bar{\omega}_{A'} \quad (15)$$

then the two levels of broken symmetry provide the graded version of the Einstein-Maxwell equations⁷:

$$\bar{\nabla}_C \bar{F}^C = 0, \quad \nabla_{C'} \bar{F}^C = 0, \quad Q = 0, \quad (16)$$

$$Q_{BB'} + \bar{F}_B \bar{F}_{B'} = 0$$

Thus, one sees that the graded Penrose transform, first envisaged by Witten^{4,8}, provides an interpretation of the field equations in terms of the breaking of graded symmetry in graded twistor space. In all existing supergravity theories, it is the torsional constraints that allow for the existence of graded null geodesics and for the construction of the transform.

It is tempting to speculate that the simplest supergravity theory, containing a complete spectrum of helicities, from -2 to 2 , derived along the above lines, may be physically viable at

Table 2 The definition of graded twistor transport

$$\begin{aligned}
\bar{D}_A \begin{pmatrix} \omega^B \\ \pi^{B'} \\ \pi \end{pmatrix} &\equiv \begin{pmatrix} \bar{\nabla}_A \omega^B - \delta_A^B \pi \\ \bar{\nabla}_A \pi_{B'} + Q_{AB'} \pi + (\bar{\nabla}_{B'} Q) \omega_A + (\bar{\nabla}_A Q_{B'}) \omega^B \\ -\bar{\nabla}_A \pi + 2Q \omega_A \end{pmatrix} \\
D_{A'} \begin{pmatrix} \omega^B \\ \pi^{B'} \\ \pi \end{pmatrix} &\equiv \begin{pmatrix} \nabla_{A'} \omega^B \\ \nabla_{A'} \pi_{B'} - 2\bar{Q} \varepsilon_{A'B'} \pi \\ -\nabla_{A'} \pi + \pi_{A'} \end{pmatrix} \\
D_{AA'} \begin{pmatrix} \omega^B \\ \pi^{B'} \\ \pi \end{pmatrix} &\equiv \begin{pmatrix} \nabla_{AA'} \omega^B - \delta_{AA'}^B \pi_{A'} \\ \nabla_{AA'} \pi_{B'} + Q_{AB'} \pi_{A'} + X_{AA'B'} \pi + X_{ABA'B'} \omega^B \\ \nabla_{AA'} \pi + Q_{AA'} \pi - (\nabla_{A'} Q) \omega_A + (\bar{\nabla}_A Q_{B'}) \omega^B \end{pmatrix} \\
X_{AA'B} &\equiv \nabla_{(A} Q_{B)A} - \varepsilon_{AB} \bar{\nabla}_A \bar{Q} \\
X_{ABA'B'} &\equiv \nabla_{(A} \bar{\nabla}_{A'} (Q_{B)B'}) + \frac{1}{2} \varepsilon_{A'B'} \nabla_C \bar{\nabla}_{C'} (Q_{B}^{C'}) \\
&\quad + \varepsilon_{AB} \varepsilon_{A'B'} (4Q\bar{Q} - \frac{1}{2} \nabla_C \nabla^{C'} \bar{Q})
\end{aligned}$$

some energy scale. If so, there would be widespread consequences for cosmology.

Finally, a point of philosophy. Local twistors survive in the superstring arena, through the theory of two-surface twistors of Penrose⁹: as the two-surface shrinks to a point, one recovers the local twistor. Is there a place in the superstring theory for a full blown twistor theory? Twistors, being fundamentally spinorial, form a 'square root' for space-time. 'Stringy' twistors ought to provide a square root for superstrings. At the quantum level they should give a 'fermionization' of the superstring theory (the reverse of bosonization)^{10,11}. Perhaps the above work should be taken as a small piece of evidence that such a theory exists.

I thank Ted Newman, Roger Penrose and John Porter for their continuing support. Supported in part by grants from the NSF and from the Alfred P. Sloan Memorial Foundation.

Received 11 September 1985; accepted 6 March 1986.

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Is there a black hole in the sky?

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The recent discovery¹ that the two quasars, Hazard 1146 + 111B,C, separated by 157 arc s, are in fact two images of the same quasar raises the question: what massive object is responsible for gravitational lensing? Ultimately, the answer will be provided by observations of a few more pairs of images of other sources when it will be possible to analyse the mass distribution of the lens. One of the imaginable candidates is a supermassive black hole. No observational or theoretical argument can be presented in favour of this possibility at present. Should the future observations point to a compact supermassive lensing object, the presence of a black hole could be established by the unique property that it would appear against the microwave background as a black spot with a diameter of ≥ 0.1 arc s.

Various candidates for a gravitational lens whose images are split by a few minutes of arc have been proposed¹, including an unusually dense cluster of galaxies, a cosmic string or a black

hole. As yet there is no observational evidence for the cluster of galaxies, and it is worthwhile exploring the consequences of the hypothesis that the lensing object is a supermassive black hole.

The angular distance between two objects with redshifts z_1 and z_2 in a universe with dimensionless density $\Omega = 1$ and cosmological constant $\Lambda = 0$ is given as²

$$D_{\text{ang}}(z_1, z_2) = \frac{c}{H_0} \frac{2}{1+z_2} [(1+z_1)^{-1/2} - (1+z_2)^{-1/2}] \quad (1)$$

where H_0 is the Hubble constant. This formula may be used to calculate the angular distance between the observer and the deflector, D_d , between the observer and the source, D_s , and between the deflector and the source, D_{ds} , as well as the effective distance to the lens, $D \equiv D_d D_{ds} / D_s$. If the source, the point-mass deflector, and the observer are perfectly aligned, then the image forms a ring with radius r_0 in the deflector's plane. This may be calculated from

$$r_0^2 = 4 GMD / c^2 = 2 r_g D \quad (2)$$

The angular radius of the ring as seen by the observer would be

$$\alpha_0 = r_0 / D_d \quad (3)$$

If the lensing object is a black hole then it might be seen as a dark spot against a bright background. For a Schwarzschild black hole, the dark spot is a circle with a diameter $3^{3/2} r_g \approx 5.196 r_g$. For a Kerr black hole, the dark spot is almost the same size, but it looks like a distorted circle³. The angular diameter of the dark circle as seen by the observer is

$$d_{\text{BH}} = 3^{3/2} r_g / D_d \quad (4)$$

Combining equations (1)-(4), we obtain for the observed diameter of the black hole

$$d_{\text{BH}} = \frac{3^{3/2}}{2} \alpha_0^2 \frac{D_s}{D_{ds}} \quad (5)$$

The angular separation between the two images of a source gravitationally lensed by a point mass is always larger than the diameter of the ring-like image, $2\alpha_0$. However, as the two images of 1146 + 111 are almost equally bright, the lens and the source may be reasonably well aligned, and I shall adopt $\alpha_0 = 157 \text{ arc s} / 2 = 3.81 \times 10^{-4} \text{ rad}$. Therefore, the expected diameter of the black spot in the microwave background becomes

$$d_{\text{BH}} = 0.078 D_s / D_{ds} \text{ arc s} \quad (6)$$

If the black hole is a relatively nearby object then $D_s \approx x D_{ds}$, and $d_{\text{BH}} = 0.078 \text{ arc s}$. If the black hole is at a cosmological distance, then $D_s > D_{ds}$, and the black spot is larger than 0.078 arc s.

Another interesting quantity is the black hole mass. For a given radius of a circular image, α_0 , it may be calculated as follows:

$$r_g = \frac{\alpha_0^2 D_d D_s}{2 D_{ds}} \quad (7a)$$

$$M = 2.3 \times 10^{15} M_\odot h_{100}^{-1} \frac{[(1+z_s)^{-1/2} - 1][(1+z_d)^{-1/2} - 1]}{(1+z_d)[(1+z_s)^{-1/2} - (1+z_d)^{-1/2}]} \quad (7b)$$

where h_{100} is the Hubble constant in units of $100 \text{ km s}^{-1} \text{ Mpc}^{-1}$. Note that at a very small observer-deflector distance D_d , the black hole mass is simply proportional to that distance:

$$M_{\text{BH}} = 8 \times 10^5 M_\odot \times D_d / 1 \text{ pc} \quad (8)$$

A black hole captures microwave background radiation with a cross-section of $27 \pi r_g^2$, and therefore it may appear as a 'source' with a negative luminosity

$$L_{\text{BH}} = -27 \pi r_g^2 \sigma [2.7 \times (1+z)]^4 \quad (9)$$

The redshift of 1146 + 111 is $z_s = 1.012$ (ref. 1). The redshift of the lens is not known. The values of the black hole mass, the

Table 1 Variation of the apparent diameter of the black spot, the black hole mass and the negative black hole luminosity as a function of the black hole redshift

z	d (arc s)	$\log M$ ($M_\odot$)	$\log -L$ ($L_\odot$)
0.001	0.078	12.36	0.98
0.01	0.079	13.35	2.99
0.1	0.092	14.36	5.15
0.2	0.11	14.67	5.92
0.3	0.13	14.87	6.45
0.4	0.16	15.02	6.90
0.5	0.20	15.17	7.30
0.6	0.27	15.31	7.70
0.7	0.37	15.47	8.13
0.8	0.57	15.67	8.63
0.9	1.1	15.97	9.33
1.0	10.8	16.97	11.41

diameter of the black spot and the black hole negative luminosity are given in Table 1 for various assumed values of its redshift, z_d , and for $H_0 = 100 \text{ km s}^{-1} \text{ Mpc}^{-1}$.

The only bright background that is present everywhere is the microwave background. The only instrument capable of either resolving the black spot, or at least noticing it as a negative luminosity source is the Very Large Array (VLA). In fact, the black-spot detection might be feasible with the present VLA instrumentation (E. L. Turner, personal communication). It would be very interesting to look for this spot if future analysis indicates the existence of a very compact supermassive object.

I thank Dr E. L. Turner for information about the discovery of the new gravitational lens before publication, and for very many stimulating discussions. Discussions with Dr M. Sikora are also acknowledged. This research was partly supported by NASA grant NAGW-765.

Received 25 March; accepted 16 April 1986.

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Is QSO1146+111B,C due to lensing by a cosmic string?

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A predicted consequence of cosmic strings is that they should produce equal-brightness double-lens images of distant quasars (QSOs), with separations of up to several minutes of arc (see refs 1, 2). Here I discuss a newly discovered lens candidate^{3–6}: QSO1146+111B,C which appears to consist of two images of equal brightness (B is 1.02 times as bright as C, with virtually identical spectra over the observed range) of a quasar at redshift $z = 1.01$, which are separated by 2.6 arc min. If this is produced by a cosmic string, its mass per unit length is $\mu \geq 4.0 \times 10^{23} \text{ g cm}^{-1}$, a value large enough to be interesting for string-assisted galaxy formation^{7–9} and near the upper limits implied by the isotropy of the cosmic microwave background^{2,10} and constraints on gravitational radiation¹¹. But note that four other QSOs, also close enough to be double-imaged, have thus far not been detected. Searches for these and for the microwave background temperature shift across the string^{2,10} would be useful.

The exact metric for a straight cosmic string has been given elsewhere²:

$$ds^2 = -dt^2 + dz^2 + dr^2 + (1 - 4\mu)^2 r^2 d\phi^2 \quad (1)$$

where μ is the mass per unit length in Planck masses per Planck length, and t, z, r and ϕ are the usual cylindrical coordinates. This is a conical space with angle deficit $D = 8\pi\mu$. Because the space-time is locally flat there is no image magnification. For convenience we adopt a value of $\Omega = 1$ for the cosmic density parameter, and the Hubble constant $H_0 = 50 \text{ km s}^{-1} \text{ Mpc}^{-1}$. The low-peculiar-velocity string produces double-lens images of equal brightness with separations²:

$$\Delta\theta = D \left[\cos \alpha - \frac{1 - (1 + z_s)^{-1/2}}{1 - (1 + z_q)^{-1/2}} \right] \quad (2)$$

where α is the angle between the string and the plane of the sky, z_q is the redshift of the QSO, and z_s is the redshift of the string at its point of closest approach. The formula applies only for those values of α, z_s, z_q which make the term in brackets positive. If 1146+111 is produced by a string, $\Delta\theta = 2.6 \text{ arc min}$ and the minimum value of μ is obtained in the limit $\cos \alpha = 1, z_s \ll 1$:

$$\mu \geq 3.0 \times 10^{-5} = 4.0 \times 10^{23} \text{ g cm}^{-1} \quad (3)$$

If this string is moving relativistically with a velocity V_s perpendicular to its own length and to the line of sight, this will produce a temperature shift

$$\Delta T \approx 2.7 DV_s/c \text{ K} \approx 2.0 V_s/c \text{ mK} \quad (4)$$

in the microwave background across the string^{2,10}. A detailed map to look for this effect would be invaluable. This effect will also cause a velocity shift $\Delta V \approx V_s D$ between the two spectra. The observations⁶ indicate $\Delta V \approx 126 \pm 309 \text{ km s}^{-1}$ in the rest frame of the QSO. A velocity shift $\approx 120 \text{ km s}^{-1}$ could be produced by a perpendicular string velocity $V_s \approx 0.5 c$. The value $\mu \geq 3 \times 10^{-5}$ already pushes the allowed^{2,10,11} upper limits so $\cos \alpha$ cannot be small and over the region of $1^\circ \times 1^\circ$ near 1146+111 $\cos \alpha \approx \text{constant}$. Thus, without loss of generality we shall adopt $\cos \alpha = 1$ in what follows.

Figure 1 shows where we should look for additional multiple QSO images. With a low-peculiar-velocity straight string as the lensing object, any QSO with redshift larger than 1.01 which has an image in the strip between the two horizontal lines must have a second image. The string itself must lie in this strip and any point within the strip must lie within 2.6 arc min of the string. Any QSO lying within 2.6 arc min of the string with $z_q > 1.01$ will be double-lensed (see equation (2)). For $0.2 \leq z_s \leq 0.6$ we would expect four of the other QSOs in the field to be double-lensed. No equal-brightness images of these QSOs have been seen. Thus, if this lensing event is caused by a string, it is surprising that there are no other easily detectable double-lensed QSOs in the field. One reason may be because of time delays between images²:

$$t_2 - t_1 = H_0^{-1} (\theta_1 - \theta_2) D [1 - (1 + z_s)^{-1/2}] \quad (5)$$

where θ_1 and θ_2 are the distances of the two images from the string. If the string passes directly down the middle of the strip then $t_1 - t_2 = 0$ for 1146+111B,C and we are guaranteed seeing them at equal brightness. The time delays for the other pairs of QSO images are, for $z_s = 0.2$ and $z_s = 0.6$ respectively: 190 yr and 6,000 yr for the quasar at $z_q = 2.22$; 240 yr and 3,900 yr for the quasar at $z_q = 2.12$; 450 yr and 1,800 yr for the quasar at $z_q = 1.10$; 300 yr and 6,500 yr for the quasar at $z_q = 1.93$. These delays are sufficient that the quasars could have varied considerably in brightness. Mini-lensing could also affect the brightness of the different images and as most of the string length is in the form of closed loops^{8,12} it is always possible that the string is significantly curved, so that it misses making images of some of these other QSOs.

If the lensing object were a black hole or cluster of galaxies (singular isothermal sphere) then the QSOs at $z_q = 2.12$ and $z_q = 1.10$ would be more likely candidates for double imaging than those at $z_q = 2.22$ and $z_q = 1.93$. As B and C are of equal brightness (B is 1.02 times as bright as C in the emission lines

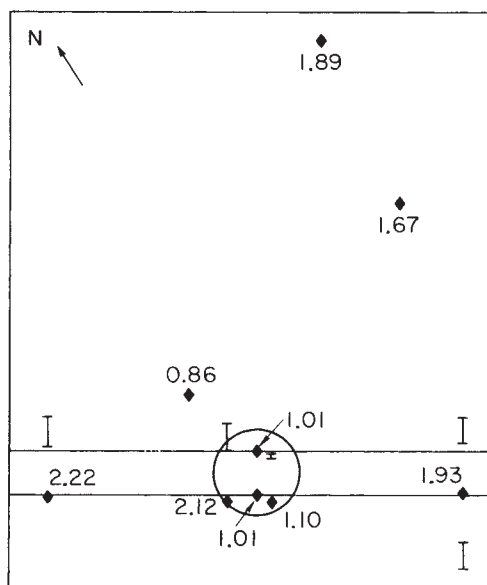


Fig. 1 Map of the known QSO images⁴ (with redshifts) in the field of 1146 + 111B,C. Images B,C are the lower and upper images respectively, with redshifts of 1.01. If the lensing object is a low-peculiar-velocity, straight cosmic string, any QSO image with $z_q > 1.01$ lying in the strip (of width 2.6 arc min) between the two horizontal lines must have an accompanying second image. If $\cos \alpha = 1$ and $0.2 \leq z_s \leq 0.6$, the possible locations for second images of four of the QSOs are shown as vertical bars. The QSO at $z_q = 1.93$ has two sets of second-image locations because the string may pass either above or below it in the figure. If the string moves with a velocity V_s perpendicular to its length and to the line of sight, the two horizontal lines, pivoting on the two QSO images at $z = 1.01$, will be rotated (A. Vilenkin, personal communication) by an angle χ , where $\tan \chi = (v_s/c) \tan \alpha$, so that over some limited ranges of χ it is possible to find solutions which require no additional QSO images. Because, as we have argued, α must be small so that μ is not too large, it is likely that this rotation is relatively small. The lower limit for μ obtained in equation (3) is uncertain (A. Vilenkin, personal communication) by a factor $K = (c - V_{s1})(c^2 - V^2)^{-1/2}$, where V is the velocity of the string and V_{s1} is its velocity parallel to the line of sight, which may be either positive or negative. If the lensing object is a singular isothermal sphere (cluster of galaxies with the requirement that its line-of-sight velocity dispersion $\sigma > 1,650 \text{ km s}^{-1}$ to produce the observed 2.6 arc min splitting¹⁴) any QSO image with $z_q > 1.01$ lying inside the circle must have an accompanying second image. If the lensing object is a black hole, any QSO image with $z_q > 1.01$ lying inside the circle must have an accompanying second image at least 1/16th as bright as it is. A failure to find any additional images in this field could be perhaps equally embarrassing for all these lensing scenarios.

which are the most likely to remain constant over a significant period of time) the black hole or singular isothermal sphere cluster must be located approximately midway between the two images, and the critical lensing angle^{13,14} is $\beta_{\text{crit}} = 1.3 \text{ arc min}$ for a QSO at $z = 1.01$. For quasars at larger redshift β_{crit} will be larger. For the singular isothermal sphere case any QSO image closer than $2\beta_{\text{crit}}$ to the centre will have an accompanying secondary image; for the black hole case any QSO image closer than $2\beta_{\text{crit}}$ to the centre will have an accompanying secondary image at least 1/16th as bright as itself. (The circle in Fig. 1 for these cases has a radius of $2\beta_{\text{crit}} = 2.6 \text{ arc min}$ (refs 13, 14). A supercluster seen edge-on, parallel to the strip and causing some image magnification is another interesting possibility as this might help to account for the unusually large number of QSOs seen⁴ in this field.

To differentiate between these lensing models and the possibility that B and C are just two different QSOs, additional spectra of B and C and searches for additional images are essential.

I thank Ed Turner for discussion results before publication. This work was supported by NASA grant NAGW-765.

Received 28 March; accepted 18 April 1986.

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Relation between solar narrow-band decimetre-wave bursts and associated X-ray bursts

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Theoretical models have been proposed, stimulated by a successful account of the jovian decametric radiation¹, for a maser mechanism, or a normal relativistic cyclotron resonance mechanism, operating in solar flares, and for transport of energy across magnetic fields to the electron gas by maser-amplified radio waves². Much effort has been directed towards observations of solar-flare radio spike bursts³⁻⁸. Here we compare solar narrow-band decimetre-wave spike bursts with corresponding X-ray events for 25 solar flares. We find that decimetre spike bursts are 10^2 - 10^3 times stronger than normal impulsive microwave bursts for the same observed amount of hard X-ray emission. This feature, along with the observation of 100% circular polarization, strongly supports a maser origin for the decimetre spike bursts.

A previous study⁸ compared narrow-band decimetre (DCM) bursts with X-ray radiation observed by the hard X-ray (HXM) and soft X-ray (FLM) detectors aboard the spacecraft Hinotori^{9,10}. In the work described here, we have taken advantage of the larger data recording capacity of the SMM spacecraft to achieve more homogeneous sampling with respect to flare intensity. We have chosen to compare the hard X-ray data at energies above 30 keV from the SMM hard X-ray burst spectrometer, HXRBS¹¹, with the radio data from the polarimeters at Toyokawa Observatory¹². The former provides hard X-ray count rate and 15-channel spectral data in the energy range 30-500 keV with 0.128-s resolution, and the latter provides radio data at four frequencies, 9.4, 3.75, 2 and 1 GHz, at a sampling rate of 0.5 s. The narrow-band DCM events treated here, for which emission is strongly polarized and is seen or strongly enhanced only at 1 GHz, are likely to be a subgroup of the fine-structure radio bursts studied by Droege³, with additional information on polarization, or part of classical DCM type IV¹³, but are probably different from the 2.65-GHz spikes investigated by Slottje^{4,5} and from blips⁶.

The data sets used in the present analysis were obtained during a period of common observations from 12 to 14 November 1981 during a so-called DCM storm. A portion of this storm is shown in Fig. 1, where the total solar radio emission is plotted against time at four frequencies for 13 November 1981. A typical spike burst is illustrated in Fig. 2, in which the 1-GHz data (a) and hard X-ray data above 30 keV (b) are plotted against the same timescale for comparison.

For each of the events studied we have obtained the peak intensity of hard X rays above 30 keV and in the radio emission at 1 GHz (Table 1). Figure 3 shows the resulting peak intensity correlation diagram for radio and X-ray events, revealing that

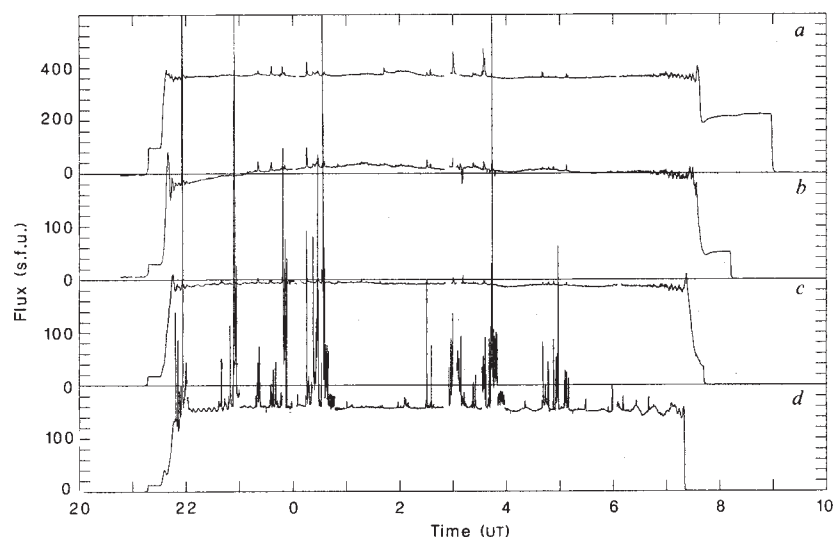


Fig. 1 Time profiles of flux at four frequencies (*a*, 9.4; *b*, 3.75; *c*, 2; and *d*, 1 GHz) observed at Toyokawa on 13 November 1981. Data gaps at 00:00, 03:00, and 06:00 UT (Universal Time) are calibration signals erased.

Table 1 Decimetre-wave events

No.	Date	Universal Time	1-GHz peak flux (s.f.u.)	HXR peak rate (counts per s)	Time delay (HXR-radio)
1	12 Nov. 1981	00:06:50	140	—	—
2		00:59:12*	877	61	-2
3		01:53:30†	74	270	25
4		02:09:19	396	—	—
5		02:32:49	15	—	—
6		03:38:20†	956	1,680	1
7		03:46:56	1,040	—	—
8		04:50:06†	40	87	2
9		06:49:32	157	—	—
10		07:08:09	154	—	—
11	13 Nov. 1981	00:15:18*	480	115	-3
12		00:32:46*	9,220	75	4
13		01:37:48†	12	58	3
14		01:38:20	20	—	—
15		01:40:16	25	—	—
16		01:58:02	30	—	—
17		03:08:32	225	—	—
18		03:22:22†	132	33	-1
19		03:24:50†	122	31	-3
20		03:34:13†	186	1,430	5
21		03:43:34*	19,300	175	8
22		04:40:27†	178	44	1
23	14 Nov. 1981	03:34:56	125	—	—
24		05:09:03	29	—	—
25		06:17:40	69	—	—

Events with no entry in the HXR peak rate column (hard X-ray peak rate above background, in the range 30–500 keV) were not observed above instrument background by the HXRBS. s.f.u. are solar flux units.

* Spike burst; † impulsive burst.

there are two distinct classes of events. One is a group of radio events for which there is very weak, if any, hard X-ray emission above 30 keV. In the second group, the radio events are associated with X-ray events of appreciable size. We will call the first group 'coherent' events and the second group 'incoherent' events. The radio bursts of the first group have the following characteristics: (1) all bursts are strongly polarized, ~100% at 1 GHz; (2) all show very spiky structure on timescales of a few seconds or less; (3) all are intense only at 1 GHz; that is, they have a narrow-band spectral character. These characteristics are a common feature of maser-associated phenomena.

The characteristics of incoherent events are almost entirely opposite to those of coherent events. Thus, the spectra of incoherent bursts are not narrow-banded in character, time profiles are less spiky, with typical time constants of ≥ 10 s, and the

degree of polarization is not high. Some incoherent bursts are almost unpolarized, and for those that are polarized the degree of polarization is much less than 100%, even at 1 GHz. It is important to note that for the incoherent events there is a linear relation between the microwave and hard X-ray intensities, reflecting the well-established proportionality between microwaves and hard X-rays^{14,15}. We should mention that there are three events (18, 19 and 22 in Table 1) which, while being classified as impulsive events, do show a slight mixture of impulsive and spike characteristics.

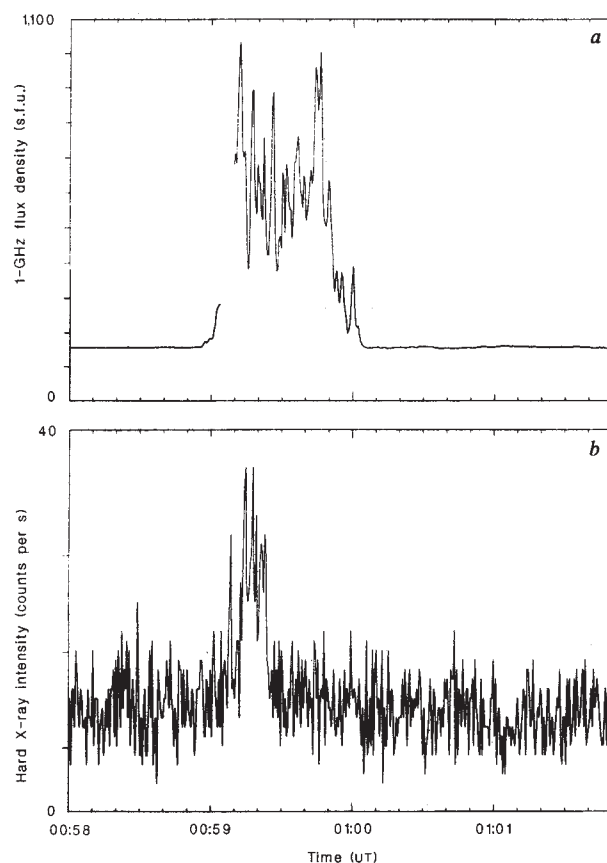


Fig. 2 High-time-resolution profiles of the 1-GHz radio burst (*a*) and X-ray burst (*b*) at 00:59:12 UT on 12 November 1981. The hard X-ray intensity is the raw count rate observed between 30 and 86 keV (including background).

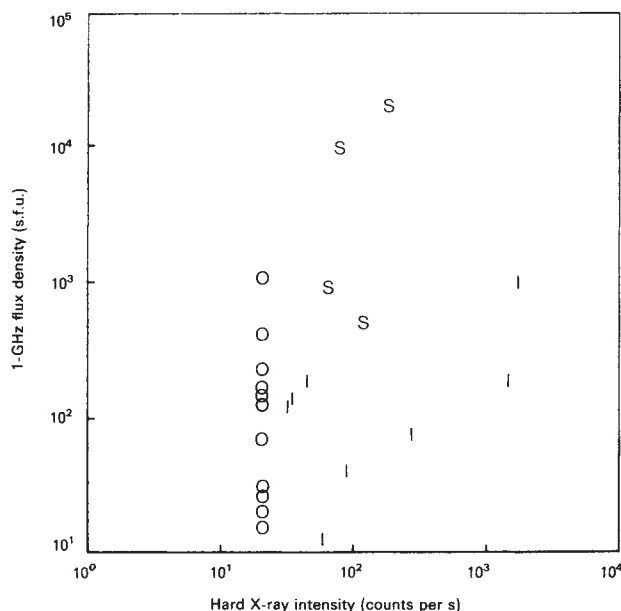


Fig. 3 Correlation diagram between peak flux at 1 GHz and X-ray intensity above 30 keV for the 25 flares studied. 'S' and 'I' indicate spike bursts and impulsive bursts, respectively. Open circles correspond to radio bursts with no observable X-ray enhancement above the background noise, so that the plotted hard X-ray intensity is a 3σ upper limit.

When one considers the above characteristics, the most striking feature of coherent events is the fact that they exhibit unusually weak X-ray emission, almost below the detection limit of the HXRBS. For the events studied here, the ratio of radio to X-ray intensity is larger by a factor of 10^2 – 10^3 for the coherent events than for the incoherent events. This enhancement factor has thus been determined observationally for the first time, and provides convincing evidence for the existence of a coherent radiation mechanism operating in these events. Its value is indispensable when one considers the saturation mechanism in the maser effect. The factor of 10^2 – 10^3 should be considered as a lower limit, because the bandwidth and the temporal structure of narrow-band coherent spike bursts might be much smaller and finer than our observational limits of 10 MHz and 0.5 s, respectively. Such bursts, if they exist, could be observed with more sophisticated instruments^{3–7,13}.

The temporal relation between the two radiations has been examined for both classes of events. Such a comparison is difficult due to the complex structure in both types of radiation. For example, detailed consideration of the event shown in Fig. 2 reveals that the identification of corresponding spikes in each data set for this burst is complicated, and a unique one-to-one correspondence cannot be specified. The results in Table 1 indicate that for those flares for which a comparison can be made there are time delays observed between the main peaks in radio and X-ray emission. In the coherent events the time delays are distributed from -3 to 8 s. Note, however, that radio bursts of the incoherent type are always delayed in time relative to the associated X-ray bursts.

Received 1 October 1985; accepted 26 February 1986.

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Bottom-current pathways in the Argentine Basin revealed by mean silt particle size

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Regional circulation patterns in the deep sea are very difficult to determine because of time constraints on the acquisition of large physical-oceanographic data sets from abyssal depths; in addition, the large number of long-term (≥ 1 yr) current-meter arrays needed to delineate bottom-current pathways are extremely costly. An alternative method for inferring benthic flow patterns, described here, is to map the mean size of the silt fraction of sediment on the sea floor. Because of the availability of a large number of core samples in the Argentine Basin, the particle-size approach provides a useful method for determining bottom-current pathways in that deep basin.

The mean particle size of the non-biogenic silt fraction of sediment from seafloor (gravity-core-top) samples has been used to delineate deep western boundary undercurrents (DWBCs) in the Vema channel^{1–3}, Amiran Passage⁴ and the lower continental rise of eastern North America^{5,6}. In those cases, however, the method was used only to identify the depth range of a high-velocity DWBC and was not applied to an entire basin, which would include areas of low bottom-current velocity. The Argentine Basin in the south-west Atlantic Ocean provides a unique opportunity to test the particle-size technique in a basin where bottom-water velocity ranges from high-velocity DWBCs to low velocities on the lower flank of the Mid-Atlantic Ridge^{7,8}. Another useful factor is the availability of a large number of core-top samples in the region.

The mean silt particle size was determined for the non-biogenic fraction of seafloor samples obtained from gravity core tops deeper than 4,000 m, in which there is no evidence of graded bedding or sedimentary structures indicative of turbidites. The bottom-current velocity inferred from particle-size data is contoured to divide the relative velocity into five divisions, arbitrarily classified from highest to lowest velocity (Fig. 1). The boundary between moderate and high relative velocity was chosen at a mean silt particle size $\phi = 6.0$ (16 μm), the mean size which delineates the top of the DWBC in both North and South Atlantic studies^{1–6}. The contour interval for relative velocity (Fig. 1) represents a difference of 0.2 in silt mean ϕ , which is analysed with a precision of 0.05 (refs 2, 3).

The inferred relative bottom-current velocity in the Argentine Basin reveals a benthic flow pattern which is controlled principally by geostrophy and bathymetry (Fig. 1). Antarctic Bottom Water (AABW) enters the South Sandwich Basin through the newly discovered Bullard Fracture Zone⁹, which has served as a bottom-water conduit throughout the Quaternary¹⁰. On encountering the South Sandwich Ridge, AABW is diverted north, where the main branch flows as a DWBC into the Georgia Basin while a minor branch flows around the eastern flank of the Islas Orcadas Rise. The two branches rejoin near a gap in the Falkland Fracture Zone¹¹, where the flow intensifies as it follows approximately the 4,000–5,000-m contour interval through the southwestern and western Argentine Basin.

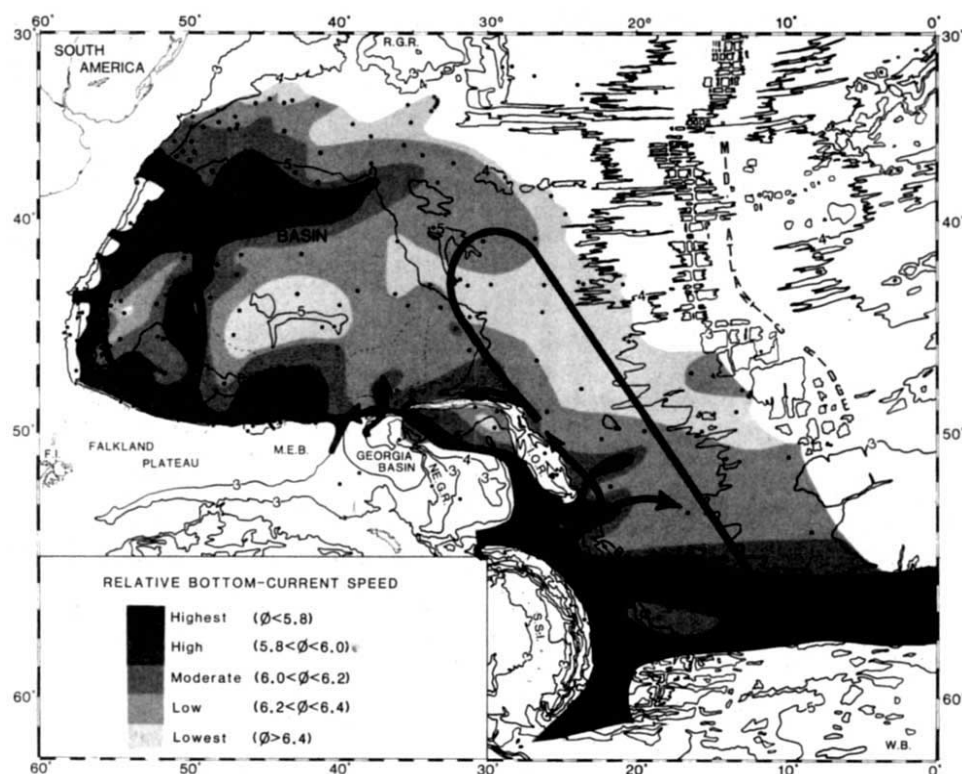


Fig. 1 The relative bottom-current velocity in the Argentine Basin region is inferred from contours of mean silt particle size, ϕ ($= -\log_2$ particle size in mm) in gravity core-top samples (dots) deeper than 4,000 m. Benthic oceanography determined from hydrocasts⁸ is shown by black arrows (width of arrow volume transport).

Bottom water leaves the Argentine Basin through the Vema and Hunter Channels¹², which have sill depths considerably shallower than the abyssal floor of the basin, with the result that the denser component of AABW, Weddell Sea Bottom Water⁷, is diverted east at depths greater than the sill depth of the outlets (Fig. 1). The axis of this flow is at depths slightly greater than 5,000 m, but water as shallow as ~4,500 m is incorporated into a broad return flow which is detectable on the lower flank of the Mid-Atlantic Ridge.

In addition to the main DWBUC flow pattern, the particle-size data reveal several abyssal gyres, or return flows (Fig. 1). A series of small gyres break away from the DWBUC adjacent to rises and ridges in the south-west part of the study area. A large gyre is seen north of the Maurice Ewing Bank and may be controlled by, or be responsible for, the topography of the crescent-shaped Zapiola Ridge¹¹. The major return-flow gyre delineated by the particle-size data is found in the western basin to the east of the DWBUC, at a depth of ~5,500 m (Fig. 1). The position of the axis of this return flow corresponds to a zone of eastward-dipping isotherms below 4,000 m which is consistent with a return flow in the hydrographic data⁸.

The particle-size data on the South American continental margin have a complex pattern (Fig. 1) resulting from the input of terrigenous material by down-slope processes through the many canyons on the slope. Some of the sources for the input of coarse silt can be seen in the seafloor data map (Fig. 1). It is possible, in principle, to change the particle-size distribution at source or to have different sources of silt particles, so that the regional pattern of particle size represents either source or bottom-current velocity variations, or both. The Argentine Basin is an excellent basin in which to test this hypothesis because of the multiple sources along the Argentine continental margin, the Falkland Plateau, and material transported by deep currents from the Weddell Sea. Despite these numerous sources, the dominant pattern of a high-velocity DWBUC with major return-flow gyres is obvious in the pattern inferred from particle-size data. Therefore, in this basin, the major factor controlling particle-size distribution appears to be the velocity of the current in which the material is deposited.

Because each geological sample represents decades to hundreds of years of sedimentation controlled by flow conditions, the relative bottom-current velocity charted in Fig. 1 represents the long-term bottom-current circulation. It is interesting to note, however, that the bottom-water circulation below 4,000 m determined from hydrocast data⁸ on a timescale of years correlates with the long-term geological evidence (decades to hundreds of years) of benthic circulation (Fig. 1). All of the major, high-velocity current patterns are mirrored in the particle-size data, including the small return-flow gyres. The only area of high current velocity which is not predicted by the oceanographic data⁸ is the broad eastward-flowing current in the northern basin (Fig. 1), but that study⁸ did not extend north of ~40°S and may have missed the eastward-flowing arm of the major gyre in the northern Argentine Basin.

The other disparity between particle-size and hydrographic data lies in the weaker eastern gyre (Fig. 1) predicted from hydrographic data⁸. The particle-size data reveal a large gyre system, with the weakest return flow in the eastern basin, but does not delineate a northern arm of eastward-flowing water in the eastern gyre.

Therefore, the major circulation pattern of long-term bottom-water flow in the Argentine Basin region can be delineated from the particle size of seafloor samples. The pattern revealed by the particle-size approach differs in only one major aspect from that obtained from hydrocast data: the predominant flow pattern includes a basin-wide return-flow gyre to the north and east rather than two separate, smaller gyres⁸. The results are consistent with a major, contour-following DWBUC in the south-western and western Argentine Basin. Because the particle-size method may be applied to existing core-top samples, it should be possible to map the major bottom-current pathways in the abyssal ocean in other areas of dense core coverage. The resulting patterns, when combined with other sediment data, should provide a necessary component for describing the sediment dynamics in areas of current-controlled sedimentation.

Research was supported by ONR contract N00014-84-C-0313 and NSF grant DPP-8316992. Curation of cores at Lamont-Doherty is provided by NSF grant DES72-01568 and ONR

Contract N00014-75-C-0210. Curation of cores at the Antarctic Core Storage Facility is provided by an NSF grant from the Division of Polar Programs. I thank F. McCoy, R. Lotti and D. Cassidy for help in obtaining samples.

Received 21 November 1985; accepted 6 February 1986.

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Ubiquitous occurrence of 2-nitrofluoranthene and 2-nitropyrene in air

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Several nitrated polycyclic aromatic hydrocarbons (nitro-PAH) are direct-acting mutagens^{1,2} and/or carcinogens²⁻⁴, and are important constituents of combustion emissions and ambient air. These nitro-PAH are emitted from various combustion sources including gasoline and diesel engine exhaust⁵⁻⁸, aluminium smelting effluent⁹, coal fly ash¹⁰, wood smoke⁶, and cigarette smoke condensates¹¹. Of these, diesel engine exhaust is the best characterized⁶⁻⁸, more than 50 nitrated polycyclic aromatic compounds having been identified by Paputa-Peck *et al.*⁸, including 1-nitropyrene (1-NP) as the single most abundant nitro-PAH. However, nitro-PAH may also be formed during source-receptor transport by atmospheric reactions of adsorbed or gas-phase PAH with oxides of nitrogen, nitric acid and other atmospherically important species such as the OH radical^{1,12-14}. Evidence for the atmospheric formation of nitro-PAH has come only recently, from observations that 2-nitropyrene (2-NP)¹⁵⁻¹⁷ and 2-nitrofluoranthene (2-NF)^{16,17} neither of which has been reported to be emitted from combustion sources, are among the major nitro-PAH present in ambient air. We present here data from several locations which demonstrate that these two atmospherically formed nitro-PAH are ubiquitous in tropospheric ambient air.

Ambient particulate matter was sampled by three different methods. An ultra-high-volume 'mega-sampler'¹⁸, with a 50% cutoff point of 20 µm and typically 6-h sampling periods, was used at Claremont, California, a site ~40 km east of downtown Los Angeles. In Aurskog, Norway, a rural residential area, an electrostatic precipitator (ESP)¹⁹ with an impact stage designed for a 15-µm cutoff was used to collect particulate matter during several weeks in the winter of 1984. The other urban-air particulate matter samples analysed were the US National Bureau of Standards (NBS) Standard Reference Materials (SRMs) No. 1648 and 1649 (refs 20, 21). These samples were collected by means of specially designed filter baghouses over a period of more than a year in St Louis, Missouri (SRM 1648) and Washington DC (SRM 1649).

High-resolution gas chromatography/mass spectrometry (GC/MS) is required to distinguish between 2-NF and the very

closely eluting 3-nitrofluoranthene (3-NF) isomer^{16,22}, as discussed below, because of this near-equality of GC retention times 2-NF has been misidentified as 3-NF in several previous studies^{15,23,24}. Our identification of 2-NF is based on the observed GC retention time, along with its characteristically low m/z 217 $[M - NO]^+$ fragment-ion abundance (~4% compared with ~46% relative abundance for this fragment ion in 3-NF)^{16,22}.

Figure 1 shows that the nitro-PAH fractions of the samples from St Louis, Claremont and Aurskog all contain 2-NF, 1-NP and 2-NP. The concentrations of these three nitro-PAH in the particles analysed from the various locations studied are given in Table 1. Unfortunately, for the NBS SRM baghouse and the Aurskog ESP samples, the actual ambient air concentrations cannot be determined because the sample air volumes are unknown. However, from our present measurements at Claremont and earlier measurements^{15,16}, these nitro-PAH are typically present in the low pg m⁻³ range. In all of the samples analysed, 2-NF was the most abundant nitro-PAH, with 1-NP and 2-NP being present in lesser amounts.

Due to the small difference in the GC retention times for 2-NF and 3-NF, the 2-NF present in SRM 1648 and in samples from Philadelphia, Pennsylvania, and a rural site in Denmark in winter were originally reported as 3-NF in refs 23, 24 and 15, respectively. These samples have since been re-analysed, with the result that 2-NF, and not 3-NF, has been shown to be present in all cases^{17,25,26}. These and our present observations show conclusively that 2-NF and 2-NP are ubiquitous in ambient particulate organic matter (POM). Although Jäger²⁷ reported

Table 1 Concentrations of nitro-PAH and BeP (µg g⁻¹) in air particulate matter samples at different locations in the USA and Norway

Compound	Claremont*	St Louis†	Washington‡	Aurskog§
2-NF	2.8	0.56	0.60	0.56
1-NP	0.36	0.16	0.20	0.15
2-NP	0.08	0.06	0.05	0.17
BeP	3.4	5.2	4.6	44
Concentration ratios				
2-NF/BeP	0.8	0.1	0.1	0.01
2-NP/BeP	0.02	0.01	0.01	0.004

* Combined sample from four 6-h sampling periods from 14 to 15 September 1985.

† NBS SRM 1648.

‡ NBS SRM 1649.

§ Sampled during February and March 1984.

|| From ref. 24.

the presence of 3-NF in ambient air in Prague, Czechoslovakia, it is possible that the nitrofluoranthene isomer identified from the thin-layer chromatography-fluorescence quenching measurements was actually 2-NF, as it is not clear that this method²⁷ could distinguish between 2-NF and 3-NF. Additionally, Morita *et al.*²⁸ have reported an unidentified nitrofluoranthene or nitropyrene isomer more abundant than 1-NP in an urban air sample in Japan; this could also be 2-NF.

Indeed, the only well-documented report of the presence of 3-NF in ambient air is that of Pitts *et al.*¹⁶, in which a small amount of 3-NF, together with a much greater amount of 2-NF, was detected in one sample collected during morning hours in Riverside, California. Based on literature data, the 1-NP and 3-NF observed in ambient POM probably originate from combustion sources⁵⁻¹¹, atmospheric transformations^{1,12,13} and/or reactions during collection^{1,29}. However, 2-NF and 2-NP have not been observed in combustion emissions, nor from any nitration reactions of adsorbed fluoranthene or pyrene in the dark^{1,29-31}, although Wu and Niki³² have recently tentatively identified 2-NP as a product of the reaction of NO₂ with monodisperse pyrene on silica particles in nitrogen in the presence of light.

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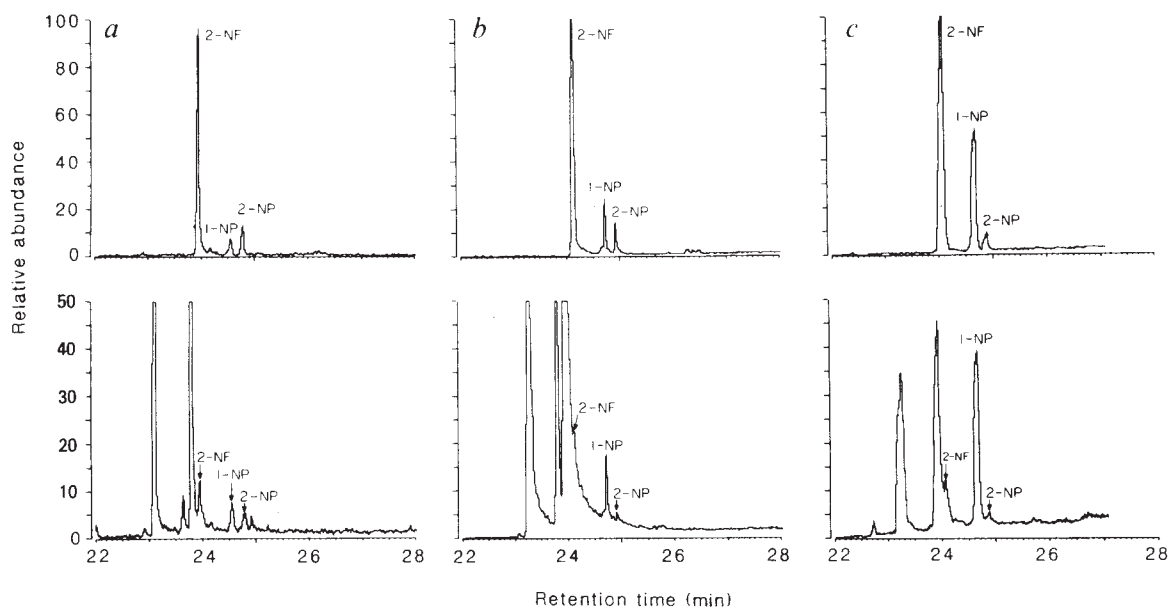


Fig. 1 GC/MS multiple ion detection (MID) mass chromatograms (top, molecular ion M^+ ; bottom, fragment ion $[M-NO]^+$) of enriched nitro-PAH fractions of extracts of air particulate matter from: *a*, Aurskog, Norway; *b*, St Louis, Missouri and *c*, Claremont, California (see Table 1). Note the characteristically low $[M-NO]^+$ fragment-ion relative abundances for 2-nitrofluoranthene (2-NF, 4%) and 2-nitropyrene (2-NP, 8%), in comparison with that of 1-nitropyrene (1-NP, 39%)^{16,22}. The samples were Soxhlet-extracted for 18 h with dichloromethane. 2-Nitrofluoranthene- d_8 and 1-nitropyrene- d_8 were added to the extracts as internal standards. The extracts were pre-cleaned by open-column silica (5% H_2O) chromatography to remove aliphatic hydrocarbons and polar material. The fractions of interest were further fractionated by normal-phase high-performance liquid chromatography (HPLC) using a semi-preparative silica column. The HPLC fractions containing nitro-PAH were analysed by GC/MS MID using a Finnigan model 3200 mass spectrometer operating in the electron impact mode. The samples were chromatographed on a 30-m DB-5 fused silica capillary column (J&W Scientific), directly eluting into the ion source. On-column injection was made at 50 °C, followed by a temperature program at 8 °C min^{-1} to 350 °C. The data were calibrated by comparing the ratios of the areas of the molecular ion (m/z 247) peaks to those of the deuterated internal standards (m/z 256)¹⁶.

Thus, 2-NF and 2-NP observed in ambient POM seem to be formed from their parent PAH during atmospheric transport^{15,16}. Table 1 lists the concentrations of 2-NF and 2-NP relative to that of benzo[*e*]pyrene (BeP), a fairly non-reactive³³ PAH of similar volatility. Although the same nitro-PAH are observed at all of the locations studied, the extent of the formation of 2-NF and 2-NP from the parent PAH varies (assuming that the ratios of emitted fluoranthene and pyrene to BeP are similar at all locations), with that for 2-NF varying much more (by a factor of ~80) than that for 2-NP (a factor of ~5). This suggests that different reaction pathways may be responsible for the formation of 2-NF and 2-NP. Table 1 also shows that the ratios 2-NF/BeP and 2-NP/BeP exhibit the same trend as the ambient temperatures; that is, Claremont > St Louis = Washington > Aurskog, consistent with the occurrence of gas-phase reactions as formation pathways for these nitro-PAH. Indeed, 2-NF is formed from the gas-phase reaction of fluoranthene with N_2O_5 (ref. 17), and this may be one reaction pathway under atmospheric conditions. Other reaction pathways which could form 2-NF and 2-NP include OH-radical-initiated reactions^{15,16} and, for 2-NP, photoinduced reactions with NO_2 (ref. 32).

Summer conditions are not necessary for the formation of 2-NF and 2-NP, as these nitroarenes are present in the winter air samples from Aurskog and Denmark^{15,26}. Indeed, the Aurskog sample contains the highest absolute amount (wt/wt particles) of 2-NP of all the samples for which data are given in Table 1. The main sources of PAH in this area of Norway are emissions from residential heating by wood and kerosene, which results in high atmospheric levels of PAH in winter (stagnant air) conditions³⁴, and as expected (Table 1), the ambient air at this location contains ~10 times more PAH (as indicated by the concentration of benzo[*e*]pyrene) than do the other samples analysed.

Thus, atmospherically formed 2-NF and 2-NP are observed in conditions ranging from Claremont in summer (high tem-

perature, high radiation flux) to Aurskog in winter (low temperature, low radiation flux). Of the known reactions of PAH under atmospheric conditions^{17,35,36}, the gas-phase reactions with N_2O_5 and/or with OH radicals followed by NO_2 addition are candidates for the formation of these NO_2 -PAH. Laboratory and ambient atmospheric measurements intended to resolve these questions are in progress.

We thank the California Air Resources Board (contract no. A4-081-32; Dr Jack K. Suder, project monitor) for financial support. The collection of the Aurskog sample was made possible by support from the Nordic Council of Ministers, through the MIL-2 project. The technical assistance of Mr Travis M. Dinoff (SAPRC) and Mr Adler Mikalsen (Norwegian Institute for Air Research, Aurskog particulate sampling) is acknowledged. T.R. thanks the Royal Norwegian Council for Scientific and Industrial Research for a 1985 Research Fellowship.

Received 30 January; accepted 10 March 1986.

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Influence of Saharan dust on the rain acidity and atmospheric input to the Mediterranean

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It has long been recognized that Saharan dust may be transported a long way from its sources, particularly over the ocean¹, and especially over the tropical North Atlantic, as far as the Caribbean^{2–7}, and over various areas of the Mediterranean^{8–10}. The eolian transport of desertic aerosols is a major contribution to oceanic sedimentation^{11–13} and may have important climatic implications¹⁴. This phenomenon is responsible for episodes of so-called 'red rain' or 'red snow' which have been identified in various areas of the European continent^{15–17}. Numerous red rains and red dust falls have been observed in Corsica. From 1984 data on the chemistry of precipitation collected in South Corsica, we show here that due to its CaCO_3 content, Saharan dust significantly increases the pH of rain water. This may counteract the effects of acidic rain. An assessment of African dust deposition indicates that, compared with the sediments discharged by the Rhône river, atmospheric input to the northwestern Mediterranean contributes significantly to sedimentation in that region.

Data were taken from our sampling station located in a clean area near the Bavella Pass (41°50' N, 9°10' E), at 1,200 m above sea level. Systematic observations on rain chemistry have been made since 1980 at this station, using a plastic gauge of 400 cm² aperture. According to the intensity of precipitation, its frequencies, duration and the accessibility of the site in winter, the sampling times ranged from several hours to one week. Generally, samples represent bulk deposition (wet plus dry), although some samples were taken within precipitation episodes and were thus not mixed with dry deposition. More rarely, in some periods without rain, dry deposition was collected separately, by rinsing the plastic gauge with distilled water. The liquid samples, which were sometimes produced from melting snow, were filtered indoors close to the field, using 0.4-µm Nuclepore filters, to evaluate the particulate matter content. The pH was measured using a potentiometric method; Na^+ , K^+ , Ca^{2+} and Mg^{2+} by atomic absorption spectrometry; and Cl^- , SO_4^{2-} , NO_3^- and NH_4^+ by a colorimetric method.

Only 1984 data will be discussed here, attention being focused on the magnitude of the load of red dust in rainwater samples and its influence on dissolved calcium enrichment and pH. Indeed, it is only the latter two parameters that are systematically affected by the incorporation of red dust in rain water. Figure 1 concerns 60 successive rain samples of 1984, including 19 'red

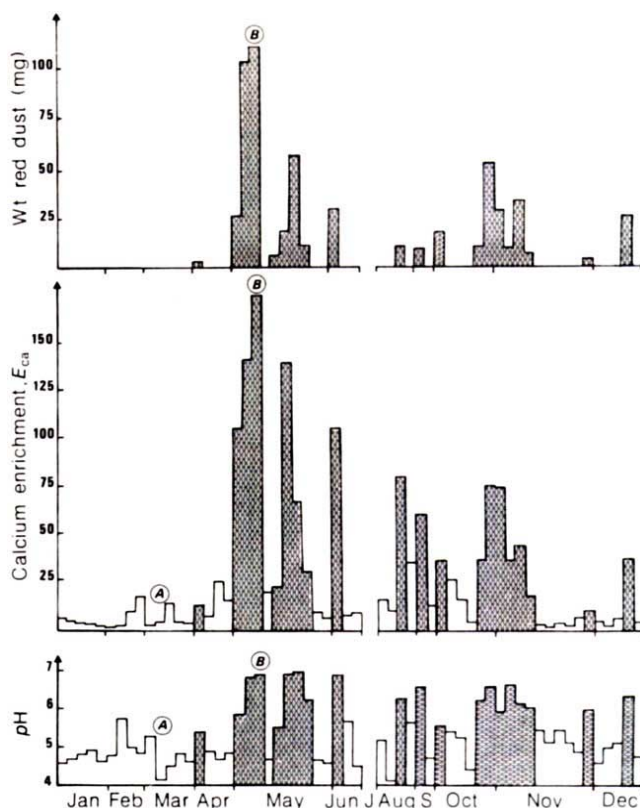


Fig. 1 Simultaneous variations in the red dust content of rainwater samples, their calcium enrichment and their pH during 1984. A and B refer to episodes for which air-mass trajectories are given in Fig. 2.

rains'. The represented weights of solid matter relate only to 'red rains', the other values being generally <2 mg per filter. The calcium enrichment, which can be considered as an index of non-marine calcium sources, is defined by the relation $E_{\text{Ca}} = (\text{Ca}/\text{Na}) \text{ rain water} / (\text{Ca}/\text{Na}) \text{ sea water}$.

Figure 1 reveals seasonal variations of the input of red dust, with sporadic episodes. A maximum is observed in spring, for example, in May when seven rainfalls account for ~60% of the annual deposition of red particulate matter, with a contribution of 40% for the two events of 5 and 10 May only. Moreover, the two observed episodes of red dust dry fallout occurred in May and June; they account for 10 and 33 mg of red dust and contribute to 8% of the bulk annual red dust input. Compared with the annual total deposition of solid particulate matter, red dust represents 84% of this flux.

Careful study of the back trajectories of the 700- and 850-mbar air masses arriving on the sampling zone confirms that this red dust originates from arid regions in the north of Africa. The incorporation into rain of desert particulate matter is accompanied by a strong increase in the dissolved calcium content. This agrees with the similar evolution of the collected load of red dust and calcium enrichment in rainwater samples. E_{Ca} is maximal and higher than 100 for some May episodes and reflects the strong influence of continental sources. Mineralogical analysis by X-ray diffraction shows that the incoming Saharan dust contains 5–30% of calcite and sometimes gypsum, although this is generally <2%. These CaCO_3 contents fit the composition of wind-blown Saharan dust collected in various regions^{18–23}. The partial dissolution of this component accounts for a significant increase in rainwater pH. During situations without input of Saharan dust, the pH varies from 4.1 to 5.6, corresponding to E_{Ca} of often lower than 10 and rarely higher than 20. Such calcium enrichments can be related to the influence of the European continental environment but it is difficult to specify whether they are due to natural sources (soils) or to industrial

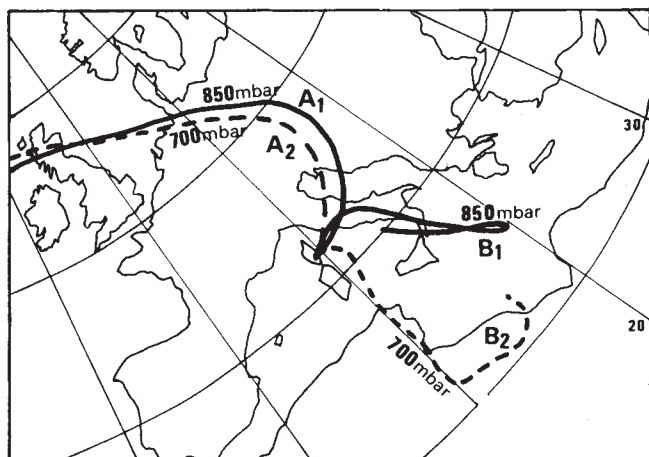


Fig. 2 Back trajectories (4 days) of air masses arriving in the sampling zone for an acidic episode (trajectories A₁ and A₂ correspond to 8 March 1984 at 18.00 UT) and for an episode with a maximal input of Saharan dust (trajectories B₁ and B₂ correspond to 10 May 1984 at 06.00 UT).

emissions (such as cement works). The influence of a residual Saharan aerosol background cannot be excluded.

The pH values increase to 6 or 7 during 13 of the 19 Saharan dust episodes. In considering the origin of the more acidic values, we must remember that rain water may be naturally acidic²⁴. In the Mediterranean Basin, the Italian volcanoes represent a major natural source of atmospheric sulphuric acidity, especially the SO₂ emissions of Etna, which constitute 1,000–2,000 tonnes day⁻¹ on average^{25–28}. Nevertheless, for the lower pH values, black particulate matter is often recovered after rain filtration. This is in agreement with the relatively high sulphate content of rain water: 5–10 mg l⁻¹ after correction of marine sulphate from bursting bubbles. According to the origin of incoming air masses, such a result could be explained by the spreading of atmospheric pollution over the northwestern Mediterranean.

The antagonistic effects of Saharan dust and other natural and industrial sources of aerosols on the acidity of the collected rain samples is supported by the data in Fig. 2. For example, we show back trajectories of 700- and 850-mbar air masses for two well-differentiated kinds of meteorological situations. The first corresponds to an episode of relatively acidic rain with a pH of 4.1 (trajectories A, 8 March), the second to a pH of 6.8 observed for the maximal input of Saharan dust (trajectories B, 10 May). Taking into account the possible atmospheric impact of long-range transport of pollutants^{29–31}, the acid episode could be related to emissions from various European industrial regions

and where there is no atmospheric mixing with African aerosols. In this case, the neutralization of rain acidity by incoming Saharan dust must occur later, after deposition, and this may have pedological and ecological implications. On 10 May, there is a more complex situation with the superposition of air masses, which could have passed over volcanic and industrial Italian regions, with air masses of African origin. Thus, the collected rain scavenged atmospheric components of different origins, and the acidity might have been neutralized within cloud and rain.

Finally, we shall consider the geochemical consequence of atmospheric deposition of Saharan material in the Mediterranean environment. If the contribution from dry fallout is included, results from 1984 at Bavella Pass indicate a red dust flux of 14 tonnes km⁻² y⁻¹, which represents an annual deposit of ~10 µm assuming a density of 1.25–1.65³². This value accounts for about 10% of the Western Mediterranean pelagic sedimentation: 100 µm yr⁻¹³³, which could be greatly influenced by marine biogenic material. How representative this flux is when evaluating atmospheric input into the Mediterranean is questionable, in that rainfall is relatively high in the mountainous areas of Corsica. With respect to the complex removal processes of aerosols^{34–35}, this effect of relief may enhance the scavenging of atmospheric particulate matter and consequently its deposition rate compared with the corresponding flux for marine-adjacent regions. However, in the case of red dust, we observed that the first rain droplets contributed to the major part of its deposition. Moreover, data from Bavella Pass (1,200 m) and the neighbouring station of Arza (700 m) show similar values for red dust deposition, collected simultaneously for 11 of the 19 red rains of 1984. For these episodes, which represent 80% of the annual red dust wet fallout in Bavella station, the input was 11% higher in Arza where the rainfall was 17% lower. Thus, the altitude effect does not seem to be very important for the removal of airborne desert material. It is, therefore, not unrealistic to extrapolate the above red dust deposition rate to sea level for the northwestern Mediterranean Basin, that is, 277,000 km². This leads to an input of 3.9×10^6 tonnes yr⁻¹, which is of the same order as the average annual downstream flow of solids in the Rhône (4.3×10^6 tonnes; ref. 36). A more precise evaluation would have to take into account regional geographical variability in the atmospheric deposition of Saharan dust. Despite the inaccuracy of the present assessment, it appears that this phenomenon must play an important part in the geochemistry and sedimentology of the marine zone considered.

This work was carried with the financial support of the CNRS Land-Sea Interactions Coordinated Group (GRECO-ICO) and ATP Aerosols désertiques and of ENSJF, Biologie-Géologie Laboratory. Air-mass trajectories were established by Météorologie Nationale. We thank Dr R. Chester for useful comments and J. A. Lefèvre and N. Kielbasa for technical assistance.

Received 5 December 1985; accepted 5 March 1986.

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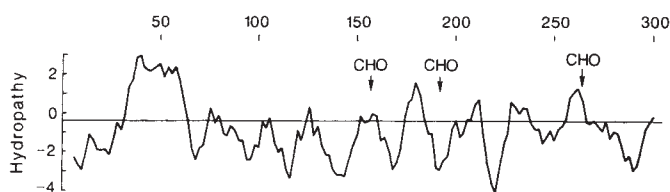


Fig. 2 Hydropathy plot of the $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ β -subunit. Nine-residue averages have been plotted using a hydropathy program based on the procedure of Kyte and Doolittle¹². The locations of potential *N*-linked glycosylation sites (CHO) are indicated.

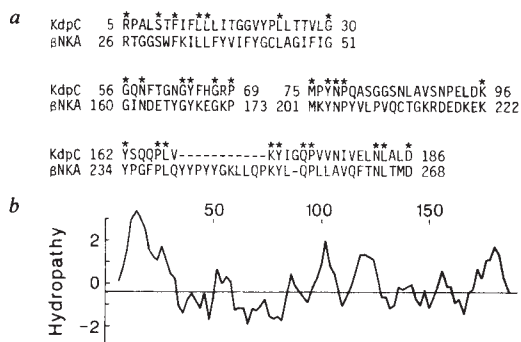


Fig. 3 Similarity between the KdpC subunit of the *E. coli* K^+ -ATPase⁴ and the $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ β -subunit. **a**, Amino-acid homology between KdpC and the β -subunit (βNKA). Homologous residues are indicated by an asterisk; gaps have been added to maximize homology. **b**, Hydropathy plot of KdpC using a 9-amino-acid window as in Fig. 2.

Photochemical labelling^{9,10} and immunochemical¹¹ procedures have led to the suggestion that the β -subunit has one or more transmembrane domains. Hydropathy analysis¹² (Fig. 2) of the deduced amino-acid sequence indicates that the protein has one transmembrane domain (residues 34–60; see Fig. 1), located near the N-terminus. Electron microscopy¹³ studies suggest that a large proportion of the β -subunit is located on the extracellular side of the membrane and Geering *et al.*¹⁴ have demonstrated that only a small cytoplasmic domain of ~2,000 daltons is removed by trypsin. Thus, the hydrophilic N-terminal domain (residues 1–33) would appear to be located on the cytoplasmic side of the membrane whereas residues 60–302 probably lie on the extracellular side. Following limited papain digestion of the dog kidney enzyme, Chin⁶ determined the partial sequence of a peptide located on the extracellular side of the membrane and approximately 9,000 daltons from the N-terminus. This sequence, Ile-?-Phe-Ile-Pro-?-?-Ile, shares partial homology with the sheep sequence beginning with Ile 87, which we have assigned to the extracellular side.

The presence of three *N*-linked oligosaccharide chains has been indicated by studies on the biosynthesis of chicken skeletal muscle $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ ¹⁵ and by endoglycosidase F treatment¹⁶ of rat kidney and axolemma $(\text{Na}^+ + \text{K}^+)\text{ATPase}$. The deduced primary structure contains three potential *N*-linked glycosylation sites¹⁷ (Asn-X-Ser/Thr), all located in the predicted extracellular regions of the protein (Asn residues 157, 192 and 264). The sequence of tryptic peptide T3 (Table 1), which begins at residue 150, could not be determined beyond residue 156 even though the quantities of the preceding amino acids in the sample being analysed were well above the limit of detection. Asparagine 157 was apparently blocked, as would be expected if it were a site for carbohydrate attachment.

It is of interest to know which transmembrane domains of the α -subunit are in contact with that of the β -subunit. Electron microscopy studies of two-dimensional crystals¹⁸ suggest that

Table 1 $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ β -subunit peptides

Tryptic peptides

T1: G K A K E E G S W K K F I W N
 T2: V A P P G L T Q I P Q I Q K ? E I A F R
 T3: F K L E W L G

N-terminal peptides

Sheep Kidney: A R G K A K E E G
 Dog Kidney: A R G K A K E E G S ? K K F I ? N
 S E K ? E F L G ? T D ? ? ? F K

$(\text{Na}^+ + \text{K}^+)\text{ATPase}$ was purified from the outer medulla of frozen lamb kidneys as described previously²². The enzyme (68 mg) was solubilized in 7% SDS, 1% 2-mercaptoethanol and two 34-mg aliquots were applied to a Bio-Gel A-5m column (2.5 × 190 cm) and eluted with 25 mM Tris-HCl pH 7.5, 0.1% SDS, 0.02% $\text{Na}_2\text{S}_2\text{O}_8$ and 1 mM 2-mercaptoethanol. The β -subunit peaks of the two column runs were pooled and lyophilized. The β -subunit (~10 mg) was dissolved in H_2O and passed through a Sephadex-G 25 column (1.5 × 90 cm) in 10 mM sodium phosphate pH 7.5, 0.1% SDS. The void volume fractions containing the β -subunit were adjusted to 5 M urea and 144 mg of solid succinic anhydride was added slowly while maintaining the pH at 7.5 ± 0.5 . After 30 min the total reaction volume was applied to a Sephadex-G25 column and eluted with 50 mM NH_4HCO_3 . Fractions containing succinylated β -subunit were pooled, adjusted to 2 M urea and concentrated by ultrafiltration to ~1 mg ml⁻¹. TPCK-treated trypsin (255 μg ; Cooper Biomedical) was added to 4 mg of succinylated β -subunit, incubated for 2 h at room temperature and lyophilized. Aliquots (2 mg) of the digest were dissolved in 0.3 ml of 1 mM dithiothreitol in HPLC grade H_2O and after 15 min adjusted to 0.1% trifluoroacetic acid (TFA) in a final volume of 0.5 ml and injected onto the HPLC system described previously²³. Peptide peaks detected by absorbance at 280 nm were partially purified on a 15-cm Vydac C₁₈ column with monitoring at 220 nm and then purified on a 15-cm Vydac C₄ column using the same solvent system (0.1% TFA in H_2O /0.1% TFA in acetonitrile). The tryptic peptides were sequenced, without prior amino-acid analysis, using an Applied Biosystems 470A gas phase sequencer; phenylthiohydantoin-amino acids were analysed on a Waters Nova-PAK C₁₈ HPLC column. Lysine was identified as succinylated lysine. The N-terminal sequences of three tryptic peptides of the β -subunit are shown. Sheep kidney N-terminal peptide data are from ref. 5, dog kidney data from ref. 6.

cleavage of the α -subunit at trypsin site 3 (see ref. 19) removes a substantial portion of the protein which is located well away from the β -subunit. The location of trypsin site 3 at Arg 262 of the α -subunit has been confirmed by amino-acid sequence analysis (L.K.L., unpublished observations). As the first and second hydrophobic domains of the α -subunit occur in the peptide that is removed, they cannot be associated with the β -subunit. It also seems unlikely that the third and fourth hydrophobic domains (H3 and H4) are in contact with the β -subunit; H3 is in close proximity to Arg 262¹ and, because of its length, H4 is likely to be located inside the transmembrane shaft and cytoplasmic stalk. Therefore, one or more of the C-terminal hydrophobic domains of the α -subunit, which begin at residue 780, are likely to be in contact with the β -subunit.

As an evolutionary relationship between the α -subunit of the $(\text{Na}^+ + \text{K}^+)\text{ATPase}$ and the KdpB protein of the *E. coli* K^+ -ATPase has been clearly demonstrated^{1,2,4}, we compared the amino-acid sequence and hydropathy plot of the β -subunit with those of the KdpC protein, a 190-amino-acid subunit of the K^+ -ATPase⁴. (An additional subunit, KdpA, is exceptionally hydrophobic and is clearly unrelated to the β -subunit.) The results suggest a possible relationship between the two proteins, although this is by no means as clear as that between the α -subunit and KdpB protein. Although there are four blocks of amino-acid homology between the two proteins (Fig. 3a), the homology is not extensive and in order to align these sequences it is necessary to postulate a number of large insertions

or deletions. Although this is reasonable considering the evolutionary time separating the two ATPases, such a procedure could also produce an apparent relationship between unrelated proteins (see ref. 20). There are also similarities between the hydropathy profile of KdpC (Fig. 3b) and that of the β -subunit (Fig. 2). KdpC has a hydrophobic domain near its N-terminal end which is likely to be a transmembrane domain, and the remainder of the protein lacks any obvious transmembrane domains. Thus, it is possible that the orientation of the KdpC protein in the membrane is the same as that of the β -subunit. Although there is insufficient evidence to conclude that these proteins have evolved from a common ancestor, the similarities suggest such a possibility. This may have important implications for other cation transport ATPases, particularly the ($H^+ + K^+$)ATPase²¹ of the gastric mucosa which, like the ($Na^+ + K^+$)ATPase and the K^+ -ATPase, transports K^+ .

We thank Dr Terence Kirley and Greg Kelso of the Peptide/Protein Chemistry Core Facility of the University of Cincinnati for analysis of peptide sequences, Jeannette Greeb and Kelly Coyne for technical assistance, Michael Hughes for synthesis of the oligonucleotide probe, Greg Wernke for DNA sequence analysis, and Gwen Kraft for preparing the illustrations. This work was supported by NIH grants PO1 HL22619 (Mission I and core 2), RO1 HL25545 and NSF Instrument Grant DMB 8414251.

Received 3 February; accepted 19 March 1986.

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Isolation of cDNA clones encoding the 20K non-glycosylated polypeptide chain of the human T-cell receptor/T3 complex

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The antigen receptor on human T lymphocytes consists of two variable immunoglobulin-like glycoproteins, α and β , which occur in association with three invariable T3 membrane proteins^{1–3}. In humans two of these proteins, T3- γ and T3- δ , are glycoproteins of relative molecular mass (M_r) 25,000 (25K) and 20,000 (20K), respectively, while the third, T3- ϵ , is a 20K non-glycosylated protein^{4–6}. On the surface of murine T cells, a non-glycosylated protein dimer composed of 17K subunits (T3- ζ) is found associated with the T-cell receptor α and β chains and the three T3-like polypeptide chains^{7,8}. It is generally accepted that major histocompatibility complex-restricted antigen recognition is a function of the α - β heterodimer. This has led to the postulation that the proteins of the T3 complex are involved in the signal transduction that immediately follows antigen recognition via the antigen receptor⁹. Events believed to be involved in early T-cell activation, such as rapid increases in phosphatidylinositol turnover and free intracellular calcium^{9,10}, can be triggered by antibodies directed against either the T3 complex or the clonotypic receptor. We have previously reported our findings on the cloning of the complementary DNA and genomic structure encoding both the human and murine 20K glycoprotein, T3- δ (refs 11–13). We now present our results on the cloning of the cDNA encoding the human 20K non-glycosylated chain, T3- ϵ .

Complementary DNAs encoding the non-glycosylated T3- ϵ chain were isolated using a high-titre rabbit antiserum, I3, directed against both T3- δ and T3- ϵ proteins. A λ gt11 library constructed from poly(A)⁺ RNA derived from pokeweed mitogen-activated peripheral blood T lymphocytes was screened for the presence of bacteriophages synthesizing β -galactosidase fusion proteins immunoreactive with I3. From the $\sim 1 \times 10^6$ plaques screened, 4 were isolated which remained positive in their reactivity with I3 throughout all further purifications.

To eliminate potential bacteriophages encoding T3- δ , DNA was isolated from the four purified plaques and analysed on a Southern blot. All four inserts were of identical size on *Eco*RI digestion and gel electrophoresis (~ 1.0 kilobase (kb)), and all hybridized with relatively equal intensity to a ³²P-labelled probe prepared from one of them (data not shown). Significantly, the T3- δ cDNA insert of clone pPGBC-9 (ref. 11) showed no hybridization with this probe. We therefore concluded that these four isolates were related clones, and since none coded for T3- δ , it was likely that they encoded T3- ϵ .

To confirm that the isolates were coding for the T3- ϵ protein, the β -galactosidase-containing fusion protein was analysed by Western blotting using monoclonal antibodies specific for T3- δ or T3- ϵ ¹⁴. The results (Fig. 1) demonstrated that bacteria containing DNA from one of the four isolates, 5a.2, synthesize a protein slightly larger than β -galactosidase which reacts only with the T3- ϵ -specific monoclonal antibody and not with the T3- δ -specific antibody. Hence, the cloned cDNA insert contained nucleotide sequences that coded for (part of) the T3- ϵ polypeptide chain.

A series of immunohistological and immunochemical analyses have led to the conclusion that the T3- ϵ polypeptide chain is expressed exclusively in T lymphocytes¹⁴. As judged by Northern blot analyses (Fig. 2a), a 1.4-kb messenger RNA hybridizes to the *Eco*RI insert of 5a.2 (subcloned into pBR322, pDJ1) only in human cells of T-cell lineage. Recent data suggest that proteins analogous to the human T3 polypeptides are associated with the murine T-cell receptor α - and β -chains^{7,8}. Figure 2b demonstrates that murine T3- ϵ mRNA, ~ 1.5 kb in size, was also found exclusively in cells of T-cell lineage. Moreover, mRNA hybridizing with the T3- ϵ cDNA has been found in all T-cell leukaemias tested so far^{15,16}.

The nucleotide sequence of pDJ1 was determined by the dideoxy chain termination inhibition method of Sanger *et al.*³².

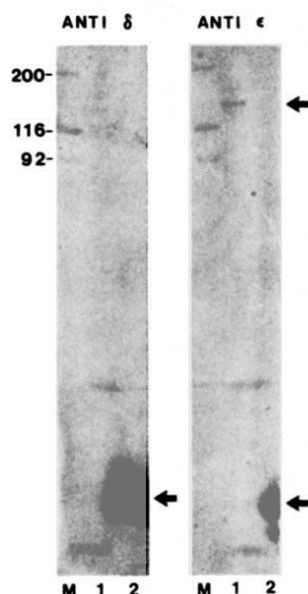


Fig. 1 Immunoblot analysis of human T3- ϵ β -galactosidase fusion protein. A λ gt11 bacteriophage isolate, 5a.2, containing a putative T3- ϵ coding region, was selected using a rabbit anti-T3- δ and -T3- ϵ antiserum. An SDS extract of 5a.2-infected *Escherichia coli* strain Y1089, synthesizing a human T3- ϵ β -galactosidase fusion protein (lane 1), and immunopurified T3 proteins (lane 2) were separated by SDS-PAGE under reducing conditions on a 7.5% gel. The proteins were transferred to nitrocellulose²⁵. The strips were first blocked with 1.5% gelatin followed by either monoclonal anti-human T3- δ antibody SP64 (left panel) or monoclonal anti-human T3- ϵ antibodies SP6 and SP10 (ref. 14) (right panel). Nitrocellulose strips were developed with ¹²⁵I-labelled sheep F(ab')₂ anti-mouse immunoglobulin (Amersham) followed by autoradiography. 'M' denotes radioactive *M_r* markers whose sizes are indicated ($\times 10^{-3}$). Lower arrows indicate positive reactivity of both anti-T3- δ and anti-T3- ϵ antibody populations with purified T3 proteins. The upper arrow indicates the presence of a T3- ϵ fusion protein slightly larger than the 116K β -galactosidase marker which was detected only by the anti-T3- ϵ antibodies.

Methods. The λ gt11 cDNA isolates were obtained as follows. The cells used for isolation of RNA were normal human peripheral blood lymphocytes that had been cultured with pokeweed mitogen for 5 days. At this time the cells were >90% T3⁺ as judged by immunofluorescence²⁶. Total mRNA was extracted in guanidine hydrochloride²⁷ and poly(A)⁺ RNA was isolated by selective retention to oligo(dT)-cellulose. Synthesis of cDNA and addition of *Eco*RI linkers for cloning into the *Eco*RI site of the β -galactosidase-encoding *lacZ* gene of λ gt11 was performed as described elsewhere^{28,29}. Before ligation into λ gt11, the cDNA was digested with *Eco*RI and fragments >800 bases were isolated from low-gelling agarose. A total of 1.2×10^6 recombinants were obtained. After amplification in *E. coli* strain Y1088, the library was grown up in *E. coli* strain Y1090 for screening with antibody. For isolation of human T3- ϵ cDNAs, an antiserum, I3, was prepared in rabbits that had been immunized with both T3- δ and -T3- ϵ proteins purified as described previously²⁰. Antibody screening was performed essentially as described in ref. 29. Nitrocellulose filters were blocked in 1.5% gelatin and then incubated with the I3 rabbit anti-T3- δ and -T3- ϵ antiserum. The filters were developed with ¹²⁵I-labelled donkey F(ab')₂ anti-rabbit immunoglobulin (Amersham). Four positive bacteriophages were plaque-purified and the DNA was isolated³⁰. To produce stable β -galactosidase fusion proteins, bacteriophage particles from one of the four λ gt11 isolates, 5a.2, were used to infect *E. coli* strain Y1089 at a multiplicity of infection of 5. Infected bacteria were grown at 30°C on nitrocellulose filters and replicate filters were prepared³⁰. The filters were baked for 2 h at 80°C, prehybridized and hybridized in 50% formamide, 5 $\times$ SSC, 50 mM sodium phosphate pH 6.5, 250 μ g ml⁻¹ sonicated salmon sperm DNA, 0.1% bovine serum albumin, 0.1% Ficoll, 0.1% polyvinylpyrrolidone, and 0.1% SDS. The *Eco*RI insert of isolate 5a.2 was ³²P-labelled to a specific activity >10⁸ c.p.m. μ g⁻¹ by the random hexadeoxyribonucleotide priming method³¹ and used to probe the filters. The filters were hybridized at 42°C overnight, then washed several times in 2 $\times$ SSC, 0.1% SDS at room temperature followed by two successive washes in 0.2 $\times$ SSC, 0.1% SDS at 65°C. Filters were exposed to Kodak XAR-5 film at -70°C using an intensifying screen. Positive colonies were selected and grown at 32°C. Induction of the *lacZ* fusion products with isopropyl β -D-thiogalactopyranoside was performed as described by Huynh *et al.*²⁹.

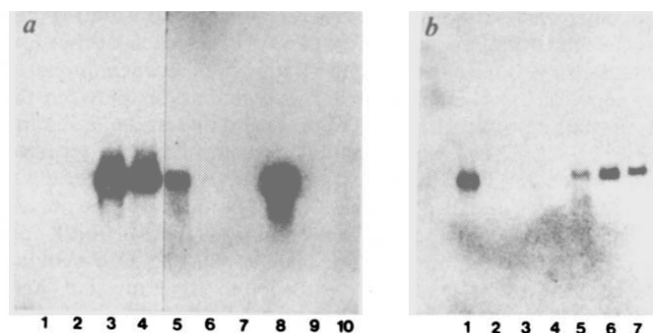


Fig. 2 Northern blot analysis of T3- ϵ mRNA. Total RNA was extracted from cells in guanidine hydrochloride²⁷. mRNA (20 μ g total) was denatured by heating to 65°C in 50% formamide and separated by electrophoresis in a 1% agarose formaldehyde gel. The RNA was transferred to Biodyne filters (ICN) and the filters were baked for 2 h at 80°C. Prehybridization, hybridization and washing steps were done exactly as described for Fig. 1 using the ³²P-labelled *Eco*RI insert from pDJ1 as probe.

Clone pDJ1 consisted of an insert of 995 base pairs (bp) with an open reading frame of 554 bp. As this clone was not full length, a second library which had been prepared from mRNA of a human T4⁺T8⁻ T-cell clone according to the method described by Okayama and Berg^{17,18} was screened using the insert from pDJ1 as a probe. From this library, pDJ4 containing an insert of ~1.4 kb, was selected for sequencing.

Figure 3b shows the complete nucleotide sequence of the T3- ϵ cDNAs. The first in-frame AUG was found at position 55 of the nucleotide sequence. There was only one long open reading frame which continued until a termination signal at position 754. A consensus poly(A) addition site, AATAAA, was found at position 1,294 followed by a poly(A) tail 14 nucleotides downstream. The relative molecular mass of T3- ϵ predicted from the protein sequence in Fig. 3b is 23,153. A hydrophobicity plot of the coding segment (Fig. 3c) predicted the presence of a putative N-terminal signal peptide containing an 11-amino-acid hydrophobic core¹⁹. This signal peptide is followed by a hydrophilic domain 104 amino acids long. A 26-amino-acid hydrophobic segment, beginning at position 105, delineated a putative hydrophobic transmembrane region. The segment between residues 131 and 190 is hydrophilic, whereas the C-terminus (191-211) is uncharged. The absence of potential sites for N-linked glycosylation in the translated amino-acid sequence was consistent with our observation that T3- ϵ contains no N-linked oligosaccharides^{5,20}.

To compare the amino-acid sequence predicted from the nucleotide sequence with that of the mature T3- ϵ protein, we performed partial N-terminal radiosequencing of purified T3- ϵ tryptic fragments. As this protein has the same *M_r* and almost the same isoelectric point as T3- δ , classic protein separation techniques failed to yield pure T3- ϵ . Because T3- δ is a glycoprotein and T3- ϵ is not, treatment with endoglycosidase F followed by preparative SDS-polyacrylamide gel electrophoresis (PAGE) had been used to obtain pure T3- δ ^{5,20}. Since Endo-F treatment does not always result in complete deglycosylation, the material that remained after digestion is often impure and can give rise to incorrect amino-acid sequences. In these experiments, therefore, we have used a different approach to obtain

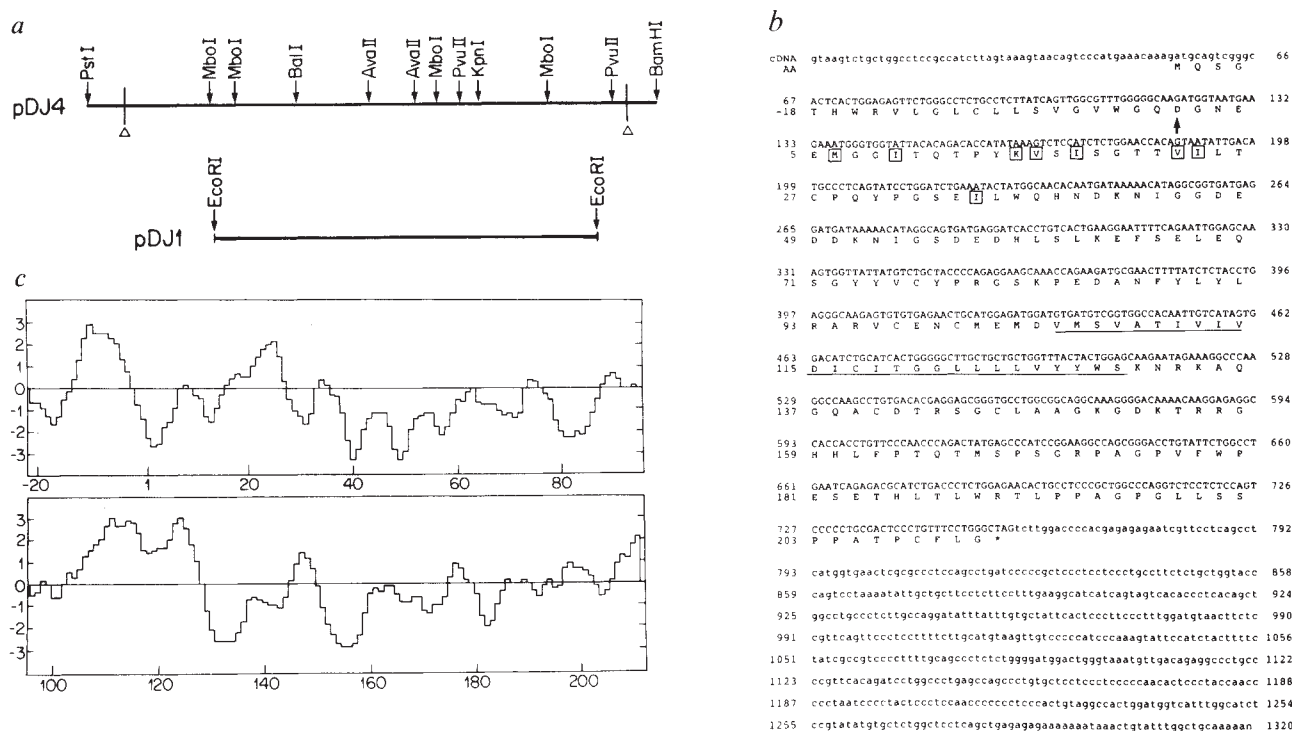
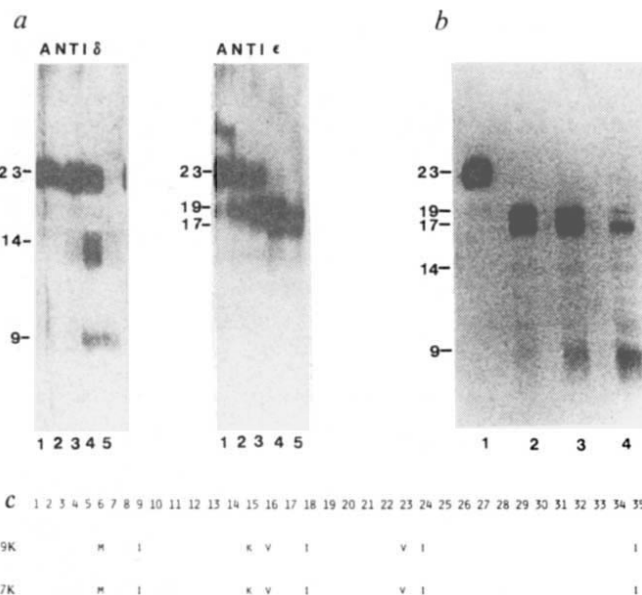


Fig. 3 Analysis of the nucleotide sequence of the human T3- ϵ chain. **a**, Partial restriction map of T3- ϵ cDNA pDJ4 containing restriction endonuclease sites used to determine nucleotide sequences. The open triangles beneath pDJ4 represent the insert boundaries. The 5' *Pst*I and 3' *Bam*HI sites are derived from the cloning vector. The *Eco*RI sites in pDJ1 are derived from linkers added during cDNA synthesis. **b**, The combined nucleotide sequence of pDJ1 and pDJ4 was determined by the dideoxy chain termination method of Sanger³² using bacteriophage M13 mp18 or mp19 (ref. 33). Isolated restriction fragments were either ligated directly into the appropriate M13 vector restriction site or made blunt-ended by treatment with the large fragment of *E. coli* DNA polymerase I before ligation into the *Sma*I site of the appropriate M13 vector. The deduced amino-acid sequence of the only long open reading frame is shown below the nucleotide sequence, beginning with the initiation methionine at position -22. The arrow indicates amino acid 1 of the mature protein. Boxed amino-acid residues 6, 9, 15, 16, 18, 23, 24 and 35 correspond to those residues determined by amino-acid sequencing (see Fig. 4c). **c**, Plot of the relative hydrophobicity of T3- ϵ . The hydrophobicity values of each amino acid have been determined using the weight system of Kyte and Doolittle³⁴. The average hydrophobicity value at residue *i* is calculated across 6 residues from *i*-3 through to and including *i*+2. Positive values indicate hydrophobic regions and negative values represent hydrophilic segments.

Fig. 4 Isolation and partial amino-acid sequences of the large tryptic fragments of the T3- ϵ protein. **a**, Western blot analyses of the trypsinization products of purified T3- δ and T3- ϵ proteins using monoclonal anti-T3- δ antibodies (left panel) or monoclonal anti-T3- ϵ antibodies (right panel). Lanes 1-5 represent titration of trypsin: lane 1, no trypsin; lane 2, 0.0015 μ g ml⁻¹; lane 3, 0.0060 μ g ml⁻¹; lane 4, 0.03 μ g ml⁻¹; lane 5, 0.15 μ g ml⁻¹. **b**, Analysis of ³⁵S-cysteine and ³⁵S-methionine metabolically labelled trypsinization products of T3- δ and T3- ϵ proteins. Lane 1, no trypsin; lane 2, 0.0015 μ g ml⁻¹; lane 3, 0.0060 μ g ml⁻¹; lane 4, 0.03 μ g ml⁻¹. **c**, N-terminal amino-acid assignments for the 19K and 17K tryptic fragments of T3- ϵ . **Methods.** Isolation of purified T3- δ and T3- ϵ proteins was as described previously²⁰. For metabolic labelling, ³⁵S-cysteine, ³⁵S-methionine and ³H-isoleucine, ³H-lysine or ³H-valine were used. Trypsinization of samples at the appropriate trypsin concentration was carried out at 37 °C for 4 h in the presence of 20 μ g sperm whale apomyoglobin (Beckman). Trypsinization was terminated by the addition of trichloroacetic acid to a final concentration of 10%. For Western blot analysis, proteolytic fragments were separated by SDS-PAGE under reducing conditions on a 15% gel. Conditions for Western blotting were exactly as for Fig. 1. For radiosequencing, proteolytic fragments were separated by SDS-PAGE under reducing conditions on a 14-20% gradient gel. The 19K and 17K bands were eluted³⁵ and radiosequenced on a Beckman protein sequencer with a cold trap modification using the Beckman 0.1 M Quadrol program 121078. Identification of the phenylthiohydantoin-amino acids was as described by Coligan *et al.*³⁶.



T3- ϵ amino-acid sequences and we believe this explains the differences between the sequence we now report and the earlier published sequence²⁰. First, both 20K T3 proteins (T3- δ and T3- ϵ) were isolated after immunoabsorbent column chromatography and SDS-PAGE. The mixture of these two proteins was subjected to limited trypsinization and large tryptic fragments (9K, 14K, 17K and 19K) were separated by SDS-PAGE. Using anti-T3- δ and T3- ϵ monoclonal antibodies in a Western blotting

experiment, we determined that the two larger fragments were derived from T3- ϵ and the smaller ones from T3- δ (Fig. 4a). The 17K and 19K fragments isolated from metabolically labelled HPB-ALL cells (Fig. 4b) had identical N-terminal amino-acid sequences (Fig. 4c). As shown in Fig. 3b, the predicted amino-acid sequence agrees with the assignment of residues 6, 9, 15, 16, 18, 23, 24 and 35 of the tryptic fragments. Therefore, the cDNA pDJ4 must encode the complete T3- ϵ protein. Since it

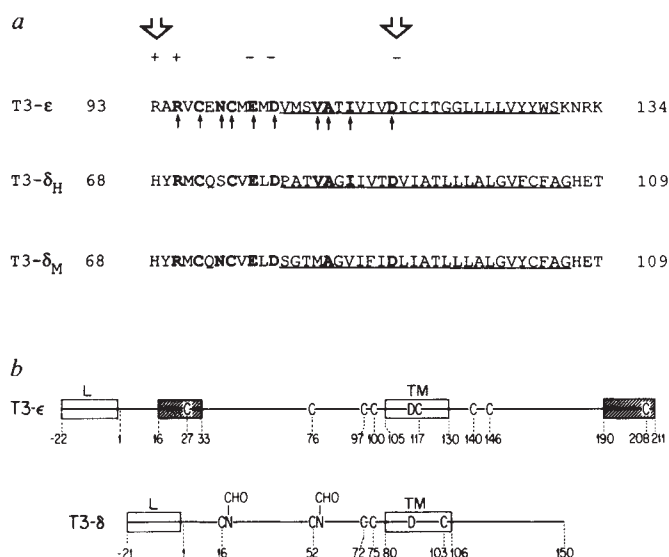


Fig. 5 Comparison of the T3- δ and T3- ϵ polypeptide chains. **a**, Homology region (see text) of T3- ϵ , including the entire transmembrane domain, compared with the human (T3- δ_H) and murine (T3- δ_M) T3- δ chains. Residues shared between T3- ϵ and human or murine T3- δ are shown in bold type and denoted by solid arrows. Boundaries of the homology region are indicated by open arrows. The transmembrane region of each chain is underlined. **b**, General features of the organization of the human T3- ϵ and T3- δ chains. L, leader sequence; TM, transmembrane region; C, cysteine; N, asparagine; CHO, N-linked glycosylation sites. The shaded areas in T3- ϵ denote the N-terminal and C-terminal uncharged regions. Numbering refers to the relative position of residues in the mature protein.

is unlikely that trypsin would have cleaved a Gln-Asp peptide bond (residues -1, 1; Fig. 3b), this sequence must represent the N-terminus of the mature T3- ϵ protein. We therefore conclude that the 17K and 19K tryptic fragments contain the N-terminus of the mature T3- ϵ protein and that amino acids -22 to -1 predicted from the cDNA sequences of pDJ4 represent the T3- ϵ signal peptide.

A search of the Los Alamos National Laboratory DNA database and the National Biomedical Research Foundation (NBRF) protein database using the NUCALN and PRTALN programs revealed no strong homologies between the T3- ϵ sequences and any other sequence. Although no strong overall homology existed between T3- ϵ and T3- δ , an interesting feature was noted. As was observed in the case of the T3- δ chain¹¹, the

T3- ϵ protein contains an Asp residue in the transmembrane region at position 115. Starting from this transmembrane Asp residue in T3- ϵ and going towards the N-terminus, there is a stretch of 23 amino acids which contains 6 charged residues. Nine of the 23 amino-acid residues are shared between T3- ϵ and T3- δ , while the remaining 14 residues contain mostly conservative changes. In addition, 5 of the 6 charged residues as well as the 2 Cys residues are shared between T3- ϵ and both the human and murine¹² T3- δ chains. In particular, the negatively charged Asp residue in the transmembrane region is of great interest because of the association of the T3 proteins with the T-cell receptor heterodimer. Both the α and β chains of the T-cell receptor contain a Lys in their respective transmembrane regions²¹⁻²⁴. The negatively charged Asp residues in T3- ϵ and T3- δ could form salt bridges with these charged residues of the heterodimer α and β chains, and such an interaction is likely to be significant because of the hydrophobic milieu of the plasma membrane. The 23-amino-acid homology region of T3- δ and T3- ϵ may also be important for the interaction with the α and β chains.

Based on the homology between T3- δ and T3- ϵ , we postulate that the region between residues 105 and 130 is a transmembrane segment and thus that the T3- ϵ protein is oriented in a fashion similar to the T3- δ chain, that is, the N-terminal domain is extracellular and the C-terminal domain cytoplasmic. Note that there are two relatively long uncharged regions, residues 16-33 and 191-211 (see Figs 3c, 5b). Although these regions neither form an α -helix nor consist of a strongly hydrophobic nucleus, they may be inserted in the plasma membrane. Further protein chemical studies will clarify this issue. Because the T3- ϵ cytoplasmic domain is considerably larger than that of the previously described α , β and δ chains, we predict that the ϵ protein must be involved in some signal transduction property of the T3 complex which is activated on engagement of the associated T-cell receptor heterodimer.

We thank Dr Ken-Ichi Arai (DNAX) for making available to us the cDNA library used to isolate pDJ4, Drs Peter van den Elsen and David Ucker for advice throughout this study, Drs C. T. Caskey and G. Rovera for help in cDNA library synthesis and screening, Sharon McGrath for preparation of this manuscript, and the DFCI/Harvard School of Public Health NIH-supported Molecular Biology Research Resource for assistance with computer analysis. This work was supported by NIH grants AI-15066 and AI-17651. D.P.G. was supported by a fellowship from the Cancer Research Institute/Eleanor Naylor Dana Charitable Trust. J.M.P. was supported by a Physician Scientist Award HD00657-01. C.L.P. is a Fellow and C.P.T. is a Scholar of the Leukemia Society of America.

Received 13 January; accepted 13 March 1986.

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Isolation of an HTLV-III-related retrovirus from macaques with simian AIDS and its possible origin in asymptomatic mangabeys

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Acquired immune deficiency syndrome (AIDS) has become a world-wide epidemic¹⁻⁶, so the development of vaccines and antiviral agents effective against the causative agent, human T-lymphotropic virus type III (HTLV-III), is vital. This work would be greatly simplified if a suitable animal model could be developed. Here we report the isolation of an HTLV-III-related retrovirus, STLV-III/Delta, from rhesus macaques (*Macaca mulatta*) with transmissible simian AIDS (SAIDS) and from asymptomatic sooty mangabeys (*Cercocebus atys*). SAIDS was initially diagnosed in several macaques previously inoculated with tissue homogenates of mangabey origin. Western blot analysis of both the mangabey and macaque sera demonstrated the presence of antibody cross-reactive primarily with the HTLV-III proteins p24 and p61. In a related experiment, analysis of these same sera revealed simian antibody to STLV-III/Delta proteins similar, but not identical, to those of HTLV-III with estimated relative molecular masses (M_r s) of 16,000 (16K), 26K, 35K, 45K, 60K and 110K. Infection of the mangabey, an African primate, with an HTLV-III-related virus may provide a clue to the origin of HTLV-III in humans. The apparent difference in susceptibility to SAIDS-like disease between infected macaques and mangabeys suggests that these species may respond differently to STLV-III infection.

Beginning with four macaques which had been inoculated with homogenates of a cutaneous lepromatous leprosy lesion from a sooty mangabey⁷, we have transmitted SAIDS and/or lymphoma by inoculation of tissue homogenates in 11 of 14 recipient macaques⁸. Co-culture of lymphoid tissue from six of these animals with the human H9 cell line led to the isolation of a retrovirus morphologically similar to HTLV-III (Fig. 1a). This retrovirus, STLV-III/Delta, induced the formation of syncytia in H9 cells and displayed a Mg^{2+} -dependent reverse transcriptase activity.

STLV-III/Delta and HTLV-III are also related antigenically. An enzyme-linked immunosorbent assay (ELISA) for HTLV-III antibodies performed longitudinally on sera from the four inoculated macaques revealed a rise in antibody titre for three of the monkeys (Fig. 2). The rise in antibody titre after inoculation suggests that these macaques may have acquired STLV-III/Delta from the mangabey. Antibody cross-reactive with HTLV-III was detected by ELISA in sera from 3 of 39 mangabeys (Fig. 3). In addition, STLV-III/Delta was isolated from several mangabeys by co-culture of peripheral lymphocytes with H9 cells (Fig. 1b).

Western blot analysis of simian antisera using detergent-disrupted HTLV-III virions confirmed the presence of HTLV-III-cross-reactive antibody in all ELISA-positive samples. Antibody specific for STLV-III/Delta was also detected in the serum of 2 macaques and 10 mangabeys that were ELISA-negative. In total, anti-STLV-III/Delta antibody was detected in sera from 5 of 11 macaques from the SAIDS transmission study and in 13 of 44 mangabeys.

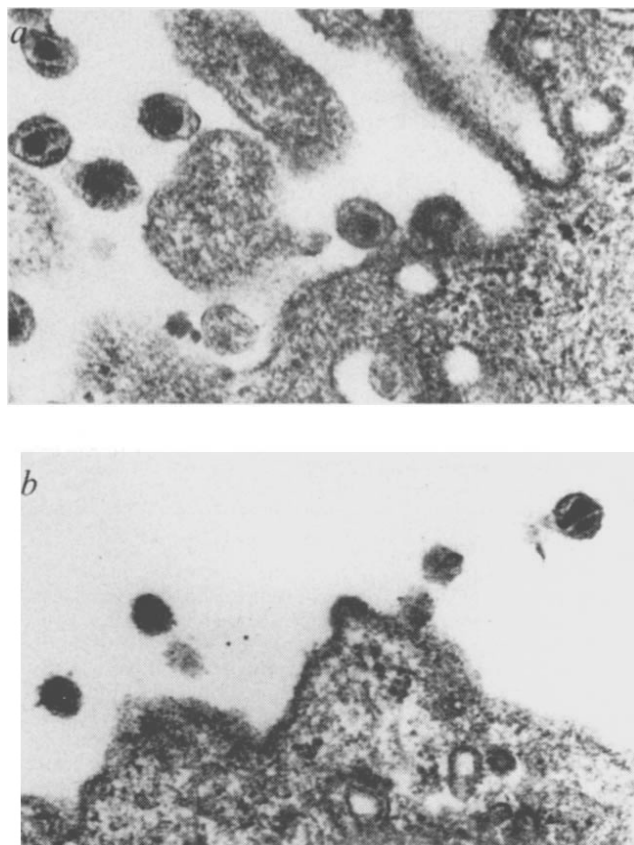


Fig. 1 Electron micrographs of the H9 human T-cell line infected with STLV-III/Delta cultured from a macaque with SAIDS (a) and from an asymptomatic mangabey (b). Ficoll gradient-purified peripheral lymphocytes from macaque B670 (a) and mangabey E038 (b) were cultured at 5×10^6 cells ml^{-1} in 7% CO_2 with $10 \mu g$ ml^{-1} phytohaemagglutinin (PHA) for 72 h in RPMI-1640 medium supplemented with 15% fetal calf serum (FCS). The PHA was subsequently removed by washing and culturing was continued in RPMI-1640 supplemented with 15% FCS and 10 half-maximal units per ml of human interleukin-2. At 3- and 7-day intervals, H9 cells (5×10^4 ml^{-1}) were added to duplicate cultures and the cells were monitored for syncytia formation. STLV-III infectivity was verified by fixed-cell indirect immunofluorescence using rhesus reference serum and electron microscopy. Infected cells were fixed in glutaraldehyde, embedded in Epoxy resin, and sectioned. Tissue sections were stained with uranyl acetate and lead citrate and photographed in a Siemens transmission electron microscope.

Final magnification: a, $\times 83,000$; b, $\times 45,000$.

Figure 4 shows Western blot results for representative sera. The predominant immunoreactive HTLV-III proteins recognized by sera from both macaque (lanes d, f) and mangabey (lanes h, j, l) were the p24 core protein and a larger protein of $M_r \sim 61K$. Additional proteins ranging in size from 35K to 50K were also variably recognized by these sera. Simian sera reacted with STLV-III/Delta proteins which were of similar, but not identical, M_r s to those of HTLV-III (Fig. 4, lanes c, e, g, i, k). The STLV-III/Delta proteins most commonly recognized by both macaque and mangabey sera were of M_r 110K and 26K; these probably correspond to the 120K *env* and 24K *gag*-related proteins of HTLV-III. Additional proteins of estimated M_r 60K, 45K, 35K and 16K also were variably recognized by the different antisera tested. Sera obtained from 50 healthy macaques and from 31 of 44 mangabeys from the Delta Regional Primate Research Center (DRPRC) colony, however, did not react with protein determinants of either virus.

The cross-reactivity of these simian antisera with both STLV-III/Delta and HTLV-III virion preparations suggests that these

two viruses have common immunoreactive epitopes. This observation was confirmed by the ability of STLV-III/Delta virions to adsorb HTLV-III-cross-reactive antibody (data not shown). STLV-III/Delta and HTLV-III are distinct, however, since in the reciprocal Western blot, a pool of human AIDS sera did not react with any of the simian virion proteins (Fig. 4, lanes *m*, *n*). In addition, Southern blot analysis of STLV-III-

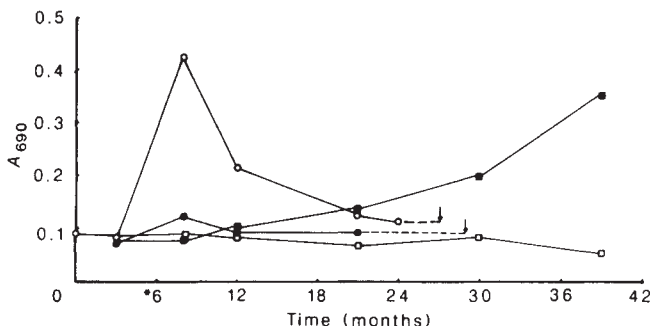


Fig. 2 Presence of HTLV-III-cross-reactive antibody in macaques inoculated with lepromatous mangabey tissue homogenates. Four macaques were inoculated as described elsewhere¹⁵ with tissue homogenates prepared from lepromatous leprosy lesions at the time designated by the asterisk. Serum was obtained from these animals at various times post-inoculation and assayed for HTLV-III-cross-reactive antibody by ELISA using the manufacturer's instructions (Litton Bionetics). The arrows mark the time of death due to SAIDS of two of the animals; two animals remained alive at this time. Monkeys are designated as follows: ○, B988; ●, 8664; ■, B845; □, 745.

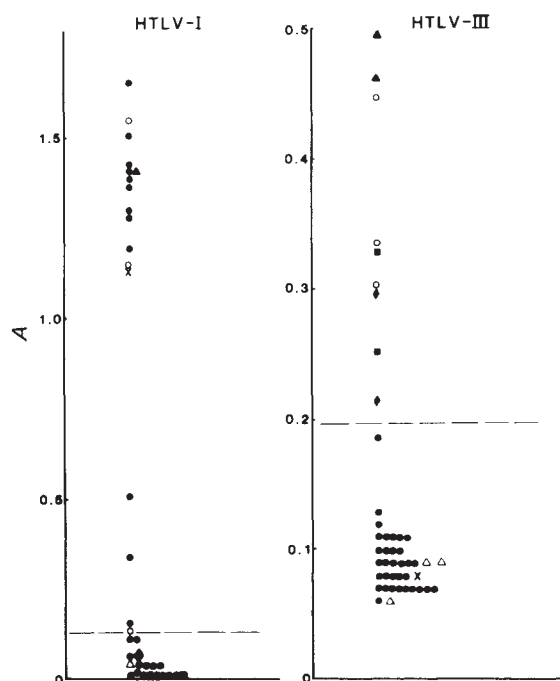


Fig. 3 Prevalence of HTLV-I- or HTLV-III-cross-reactive antibody in the DRPRC mangabey monkey colony. Random sera obtained from mangabey monkeys at the DRPRC were assayed by ELISA (Litton Bionetics) for antibodies to either HTLV-I or HTLV-III. The dashed lines designate the cut-off point for significant positive values calculated for human samples as described in the assay. Individual samples of interest are designated as follows: ○, positive human control sera; △, negative human control sera; ●, representative mangabey sera. The rest are sera obtained at various time intervals from mangabey monkeys A015 (×), D089 (▲), E038 (■) and D178 (◆).

infected H9 cell DNA using radiolabelled sequences complementary to HTLV-III⁹ failed to reveal genetic homology between the two viruses, even under conditions of low stringency (data not shown).

STLV-III/Delta is antigenically distinct from the HTLV-I-related simian virus STLV-I¹⁰. Analysis of mangabeys for anti-HTLV-I antibodies by ELISA specific for the viral p24 core protein detected significant antibody in 14 of 32 monkeys tested (Fig. 3), whereas sera from sick macaques were uniformly negative for anti-HTLV-I antibody (data not shown). Antibody cross-reactive with HTLV-III was detected in several healthy mangabeys as well as in sick macaques. However, there was individual variation, with several mangabeys being seropositive for a single virus. Thus, Western blot analysis of serum from mangabey A015, which is seropositive for HTLV-I but seronegative for HTLV-III by ELISA, did not detect antibody to STLV-III/Delta viral proteins (data not shown).

A macaque retrovirus similar to STLV-III/Delta, STLV-III_{Mac}, has been identified in association with experimentally induced SAIDS at the New England Regional Primate Research Center (NEPRC)^{11,12}. The putative *env*- and *gag*-related proteins of this virus, however, have identical *M_s* to those of HTLV-III; antibody cross-reactive with the NEPRC virus was also detected in human AIDS serum. The disparity in cross-reactivity observed between that study and our own may reflect differences in the techniques used. The difference observed in the *M_s* of the putative core proteins, however, may be significant, particularly as in both studies the simian viral proteins were normalized to those of HTLV-III. In our hands, Western blot analysis also detected other STLV-III/Delta proteins which

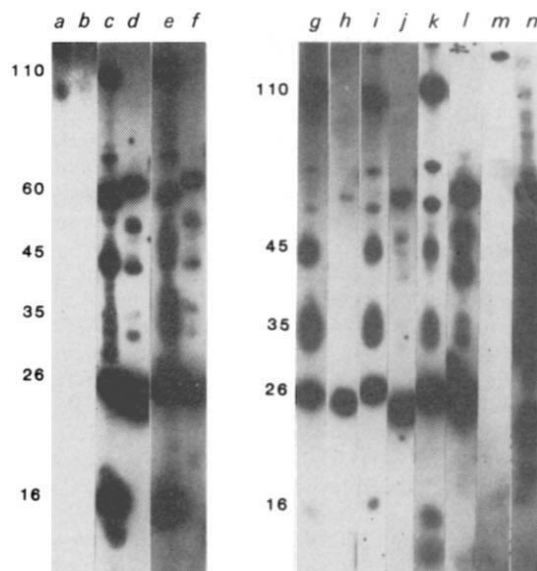


Fig. 4 Western blot analysis of the reaction of primate antisera with detergent-disrupted STLV-III/Delta and HTLV-III virion proteins. STLV-III/Delta virions were purified from infected H9 cell culture supernatants as described elsewhere¹⁶⁻¹⁸. HTLV-III virions were obtained on a contractual basis from the NIH. STLV-III/Delta (lanes *a*, *c*, *e*, *g*, *i*, *k*, *m*) and HTLV-III (lanes *b*, *d*, *h*, *j*, *l*, *n*) virions were treated with SDS and 2-mercaptoethanol, boiled briefly and subjected to electrophoresis through a 10% polyacrylamide gel containing SDS as described elsewhere¹⁸. Size-fractionated proteins were electroblotted onto nitrocellulose and the transferred immunoreactive proteins detected by incubation with primate antiserum. Antigen-antibody complexes were detected by subsequent incubation with ¹²⁵I-labelled *Staphylococcus* protein A, followed by autoradiography. Lanes *a*, *b*, an over-exposure of normal rhesus control sera; lanes *c*, *d*, rhesus B988 serum; *e*, *f*, rhesus B845 serum; *g*, *h*, mangabey E038 serum; *i*, *j*, mangabey D178 serum; *k*, *l*, mangabey D089 serum; *m*, *n*, pool of human AIDS sera.

had similar, but not identical M_s to those of HTLV-III. Taken together, these data suggest that STLV-III/Delta is related to, but distinct from, STLV-III_{Mac}. Resolution of this discrepancy will require a direct comparison of the two viruses.

An HTLV-related virus endemic in our mangabey colony in the apparent absence of SAIDS is notable. SAIDS at DRPRC has been confined to a single series of transmissions which originated with a mangabey tissue inoculum. This fact, coupled with the prevalence of the virus in mangabeys, suggests that STLV-III/Delta in our macaques with SAIDS is of mangabey origin. Although STLV-III appears to be endemic in our mangabey colony, the characteristic SAIDS pathology has not been observed among these animals. These observations suggest that virus transfer from an African (mangabey) to an Asian (macaque) species may result in significant differences in pathogenicity. A comparison of host responses to infection in the two species may prove useful in understanding the pathogenesis of human AIDS.

STLV-III has also been reported in the African green monkey^{13,14}. The presence of HTLV-III-related retroviruses in at least two African primates, the African green monkey and the mangabey, is consistent with the hypothesis that HTLV-III

emerged in humans as a result of an early trans-species transfer in Africa.

We thank N. Meiners, C. Wall and P. Eddy for technical assistance. This work was supported in part by a grant from the Division of Research Resources, NIH, and by USPHS grants from the NCI.

Received 2 December 1985; accepted 13 March 1986.

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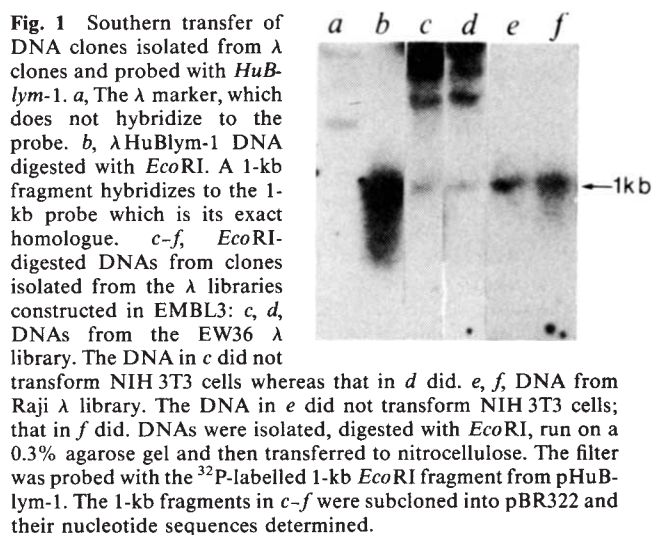
Mechanism of activation of *HuBlym-1* gene unresolved

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The *HuBlym-1* gene has been isolated from Burkitt's lymphoma DNA on the basis of its reported ability to produce foci of cells when NIH 3T3 cells are transfected with it^{1,2}. The sequence of *HuBlym-1* has been reported¹ but it has not been established whether the sequence of the transforming gene differs from that of the gene in normal tissue, as might be expected from the common observation of mutational activation of proto-oncogenes into oncogenes. I report here that there are no differences between the sequences of three recombinant DNA clones that were isolated from Burkitt's cell lines and that cross-hybridize to *HuBlym-1* but do not induce foci when transfected into NIH 3T3 cells, and the reported sequence of Burkitt's lymphoma *Blym*³. Also there is no obvious or consistent increase in the transcription of the *HuBlym-1* gene in Burkitt's lymphoma cell lines of the type that might otherwise have accounted for its transforming activity even in the absence of a mutation. How the *HuBlym-1* gene is activated therefore remains a mystery.

Total genomic DNAs isolated from six Burkitt's lymphoma cell lines have been reported to induce foci with an efficiency of between 0.1 and 1.0 transformants per μ g DNA when transfected into NIH 3T3 cells². The gene responsible for this transforming ability, *HuBlym-1*, has been cloned from the Burkitt's lymphoma cell line CW678 (ref. 2). This cloned gene transforms NIH 3T3 cells with high efficiency and has the same pattern of restriction enzyme sensitivity in a transfection assay^{2,4} as the genomic DNA from all six Burkitt's lymphoma cell lines². The biologically active transforming gene is contained within a 1.0-kilobase(kb) *EcoRI* fragment of the clone². This fragment has been subcloned into a plasmid, pHuBlym-1, and its nucleotide sequence determined³. The gene is 600-700 nucleotides long and is apparently interrupted by a single intervening sequence, yielding a predicted polypeptide of 58 amino acids³. Previous, though not extensive, studies indicate that the *HuBlym-1* gene is neither amplified nor rearranged in Burkitt's cell lines³, there-



fore other possible mechanisms of activation of the *HuBlym-1* gene have to be considered. It is known that *ras* genes can be activated to a transforming phenotype by a single base mutation at position 12 or 61 (refs 5, 6), or by coupling to a viral long terminal repeat (LTR) which gives enhanced transcription of the gene^{7,8}. Thus, two possible mechanisms for the activation of the *HuBlym-1* gene are changes in its nucleotide sequence and changes in the level of transcription of the gene.

During the original isolation of λ HuBlym-1, a number of other clones were isolated which hybridized to the *ChBlym-1* probe^{2,9}; these clones were screened together with λ HuBlym-1 in a transfection assay. λ HuBlym-1 was selected because it transformed NIH 3T3 cells whereas 14 other clones did not (A. Diamond, personal communication). As these other clones could contain the non-transforming counterpart of the *HuBlym-1*, they have now been analysed by digestion with *EcoRI*, gel electrophoresis and Southern blotting to identify those that contain a DNA insert similar to that in λ HuBlym-1.

DNAs from six of the clones showed restriction enzyme digestion patterns similar to that of λ HuBlym-1, including the presence of a 1.0-kb *EcoRI* fragment which in λ HuBlym-1 contains the active transforming *HuBlym-1* gene² (Fig. 1b). The 1.0-kb fragment from one of the clones was subcloned in pBR322 and the nucleotide sequence of both strands was determined, using a combination of the dideoxy chain termination method¹⁰

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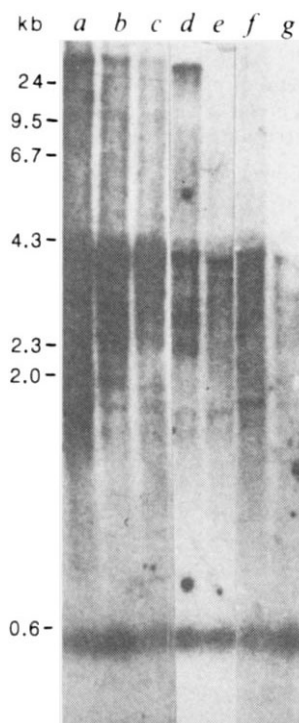


Fig. 2 Hybridization of *HuBlym-1* probe to poly(A)⁺ RNA. *a-c*, RNAs isolated from Burkitt's lymphoma cell lines CW678, EW36 and Raji, respectively. *d, e*, RNAs isolated from the EBV-immortalized cell lines LAZ ϕ 156 and LAZ 388. *f*, RNA from a pre-B-cell line; *g*, RNA from a myeloma cell line. The size markers shown are λ DNAs digested with *Hind*III. The arrow indicates the *Blym* band at ~600 bases. Total RNA was isolated, poly(A)⁺ RNA selected and samples (10 μ g) of poly(A)⁺ RNA run on a 1.5% formaldehyde gel. The RNA was then transferred to a nitrocellulose filter, which was hybridized to ³²P-labelled plasmid pHuBlym-1.

and chemical sequencing¹¹. The nucleotide sequence observed was exactly the same as that already determined for *HuBlym-1*. This result was completely unexpected. In order to confirm it, recombinant DNA libraries were constructed from the DNAs of two other Burkitt's cell lines: EW36, an American Epstein-Barr virus (EBV)-negative line¹², and Raji, an African EBV-positive line¹³. These lines were chosen as they represent both associated and non-associated EBV lines¹⁴ and because they are clearly of different origin. Both have been shown to transform NIH 3T3 cells. The DNAs of these cells were partially digested with *Mbo*I, size-fractionated and ligated into the *Bam*HI site of the λ vector EMBL4. After *in vitro* packaging, the library was screened with the 1.0-kb *Eco*RI fragment from pHuBlym-1 and plaques hybridizing to this probe were selected. Both intact phage and DNA were separately used to transfect NIH 3T3 cells. Two subsets of clones were picked from each of the libraries: those that induced transformation of NIH 3T3 cells and those that did not. DNAs from the two groups were then screened for the presence of a 1.0-kb *Eco*RI fragment (Fig. 1c-f). Four clones which contained a 1.0-kb *Eco*RI fragment were selected: one from each of the two Burkitt's libraries that transformed NIH 3T3 cells and one from each of the two that did not transform NIH 3T3 cells. The 1.0-kb fragment from each clone was subcloned into pBR322. In this case also, the nucleotide sequence of the two transforming and one of the two non-transforming clones were shown to be identical to the sequence of the original transforming *HuBlym-1* (approximately 60% of the other non-transforming gene was sequenced and found to be identical).

All the genes described and sequenced so far in this paper were isolated from DNA obtained from Burkitt's lymphoma cell lines. It was therefore important to clone and isolate the corresponding DNA sequence from a recombinant DNA library prepared from a non-Burkitt's lymphoma cell line. DNA was therefore isolated from the IMR 90 cell line¹⁵, which is a human embryo fibroblast line that is not immortalized but which grows well for 20-30 passages¹⁵. The recombinant DNA library was prepared and screened as above except that the lambda vector used was λ 47.1. Two clones homologous to the 1.0-kb *Eco*RI fragment probe isolated from pHuBlym were selected, their 1.0-kb *Eco*RI fragments were subcloned and the nucleotide sequences of both strands determined for one of the subclones.

Again, the sequences were identical to that of *HuBlym-1*.

The level of transcription of *HuBlym* in several Burkitt's lymphoma cell lines and in several other human cell lines was investigated by Northern transfer analysis (Fig. 2). In all the lines examined, a messenger RNA of ~600 bases was detected at a similar level, indicating that the induction of foci by DNA from Burkitt's lymphoma cell lines is not a consequence of increased transcription of the *HuBlym-1* gene.

The nucleotide sequences of the three non-transforming alleles of *HuBlym-1* described here are identical to the sequence reported for the allele that induced focus formation in NIH 3T3 cells³. The results of the mRNA analysis demonstrate that increased levels of *HuBlym* mRNA are not detected in Burkitt's lymphoma cell lines, however there is always a problem in knowing what to use to make a proper comparison. The way in which the *HuBlym-1* gene is activated therefore remains an open question.

I thank Dr Geoffrey Cooper for his hospitality during this work and for his willingness to read the assays for focus formation that were part of these experiments. I also thank Dr John Cairns for stimulating discussions and helpful comments on the manuscript. J.M.D. was supported by a Travelling Fellowship from the ICRF, London and by a special Fellowship from the Leukaemia Society of America.

Received 5 November 1985; accepted 28 February 1986.

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COOPER AND DIAMOND REPLY—The work reported by Dr Devine was in part performed in this laboratory and was to have been completed here following her departure in October 1984. At that time, transfection experiments were to be performed on the clones isolated from normal human DNA and the sequence of the clone from normal human DNA required completion to resolve several existing ambiguities and gaps. However, we found that all of the clones from the Raji and EW36 Burkitt's lymphoma libraries used by Devine contained cellular inserts whose restriction endonuclease cleavage patterns were indistinguishable from each other and from the original isolate of λ HuBlym-1. Since these clones were isolated from libraries constructed with DNAs partially digested with *Mbo*I, these results strongly indicated the possibility of contamination of Devine's isolates with the original transforming clone. In addition, transfection assays performed and read by Devine before she left the laboratory indicated that the clones designated by her as "non-transforming" from the CW678 and EW36 Burkitt's lymphoma libraries induced transformation with efficiencies comparable to the clones designated "transforming". Restriction digests of the clones isolated from a random library of normal human DNA were also found to be identical to the original λ HuBlym-1 isolate, again raising concerns about contamination. We therefore concluded that a bona fide normal copy of the gene had to be isolated and analysed in order to exclude the possibility of contamination of the clones on which Devine has reported.

A clone of the 5-kilobase (kb) *Hind*III fragment containing *Blym-1* was therefore reisolated from normal human DNA. The restriction map of this clone is different from other *Blym-1*

clones in the laboratory, ruling out the possibility of cross-contamination. The 1-kb *EcoRI* fragment of this clone induces transformation of NIH 3T3 cells with efficiencies comparable to that of the 1-kb *EcoRI* fragment of the original λ HuBlym-1 clone. Both of these clones have also displayed comparable activities in assays of transformation of primary human foreskin fibroblasts to anchorage independence and transformation of BALB 3T3 cells to platelet-derived growth factor-independent growth. Further, the nucleotide sequences of the 1-kb *EcoRI* fragments of the normal and Burkitt's lymphoma clones are identical.

We therefore conclude that activation of transforming potential does not require a mutation within the minimal 1-kb transforming region of *Blym-1*. These results suggest the hypothesis that flanking sequences may regulate transforming activity and experiments are now in progress to test this possibility. The notion that regulatory changes may be involved in activating transforming genes is not inconsistent with the fact that there are no significant increases in the total level of expression of *Blym-1* in Burkitt's lymphomas compared with Epstein-Barr virus-immortalized cell lines. In fact this situation is similar to that observed with *myc*, where its activation by translocation appears to result in de-regulation of gene expression rather than in a significant increase in *myc* RNA levels in most Burkitt's lymphomas and plasmacytomas¹⁻⁴.

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Specific inhibition of herpesvirus ribonucleotide reductase by synthetic peptides

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Ribonucleotide reductase is an essential enzyme for DNA synthesis in all prokaryotic and eukaryotic cells; it catalyses the reductive conversion of ribonucleotides to deoxyribonucleotides. Several herpesviruses including herpes simplex virus type 1 (HSV-1)¹, HSV-2², pseudorabies virus (PRV)³, equine herpesvirus type 1 (EHV-1)⁴ and Epstein-Barr virus (EBV)⁵ have been found to induce novel ribonucleotide reductase activities. There is evidence that the HSV-1 ribonucleotide reductase activity is virus-encoded⁶ and essential for virus replication⁷. This makes herpesvirus ribonucleotide reductases potential targets for antiviral chemotherapy. The HSV-1-encoded enzyme consists of two subunits^{8,9,18}: V136, the large subunit of relative molecular mass (M_r) 136,000 (136K) (RR₁), which has been shown to be essential for enzyme activity⁷,

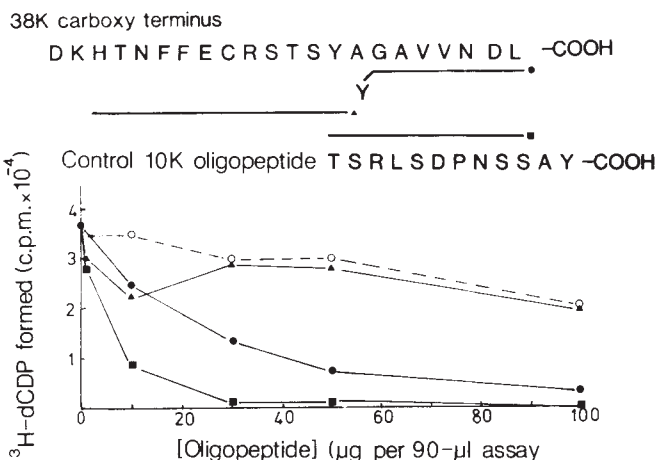


Fig. 1 Effect of synthetic oligopeptides on ribonucleotide reductase activity in an extract of HSV-1-infected baby hamster kidney (BHK) cells. The figure shows the effects of increasing concentrations of peptides 1-4 (●, ○, ■ and ▲, respectively). DK...DL-COOH represents the 22 amino acid sequence of the 38K carboxy terminus. Horizontal lines indicate the amino acid sequences of peptides 1, 3 and 4.

Methods. Synthetic oligopeptides were obtained from Cambridge Research Biochemicals (Cambridge, UK) and Peptide and Protein Research Consultants (Windsor, UK). Crude extracts were obtained from virus-infected cells as described elsewhere⁶ and partially purified by precipitation with 45% ammonium sulphate. Ribonucleotide reductase was assayed by determining the amount of cytidine diphosphate (CDP) converted to deoxycytidine diphosphate (dCDP) in a 90-μl reaction (for 1 h at 31 °C) containing 100 μg partially purified extract, 200 mM HEPES pH 8.2, 10 mM dithiothreitol (DTT), 100 μM CDP and 1 μCi ³H-CDP (10-30 Ci mmol⁻¹). The nucleotides were converted to the corresponding nucleosides by digestion with snake venom phosphodiesterase and the resulting cytidine and deoxycytidine separated by reverse-phase HPLC (B.M.D., in preparation). In the conditions of this assay, the host cell enzyme is inactive and only the viral enzyme contributes to the measured activity.

and V38, the small subunit (RR₂) which forms a complex with the large subunit and is also likely to be essential for enzyme activity⁹. Two particular features of the enzyme make it an attractive antiviral target. First, there is evidence for a common, highly conserved herpesvirus ribonucleotide reductase and second, the interaction between the large and small subunits may itself be exploitable. Here we identify a synthetic peptide which specifically inhibits the activity of virus-induced enzyme. We deduce that the mechanism of inhibition involves interference with the normal interaction between the two types of subunit.

The discovery of an oligopeptide which inhibits ribonucleotide reductase activity arose from experiments designed to show that both RR₁ and RR₂ were necessary for enzyme activity. To this end, RR₂ was removed from an enzyme extract of HSV-1-infected cells by immunoaffinity chromatography using IgG from serum raised against the synthetic octapeptide NH₂-Tyr-Gly-Ala-Val-Val-Asn-Asp-Leu-COOH (YGAVVNDL, using the one-letter code). This peptide corresponds to the carboxy-terminal seven amino acids of RR₂ plus an amino-terminal tyrosine added to facilitate linkage to a carrier protein⁹. Immunoaffinity chromatography was performed by first adsorbing the enzyme extract with immune anti-peptide IgG immobilized on Sepharose 4B. After stringent washing, bound RR₂ was eluted with the synthetic peptide. However, attempts to reconstitute the enzyme activity by adding back purified RR₂ were unsuccessful at least because the synthetic peptide necessarily present with eluted RR₂ inhibits enzyme activity (see below). Frame *et al.*⁹ had previously obtained indirect evidence that the carboxy terminus of RR₂ is masked by the large subunit; this suggested that the synthetic peptide might correspond to a region

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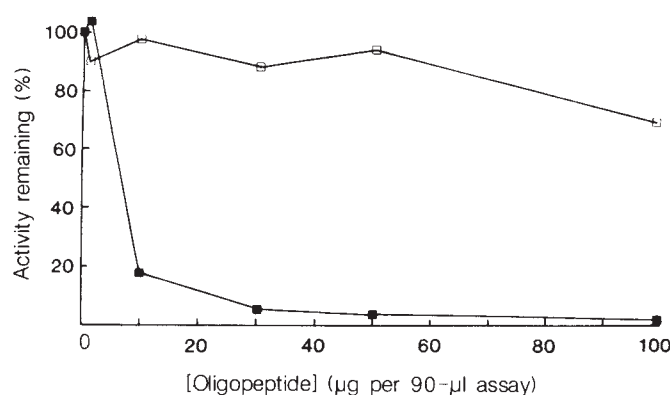


Fig. 2 Inhibition by the synthetic peptide 3 (see Fig. 1 and text) is specific for the viral and not the cellular ribonucleotide reductase. Partially purified extracts were obtained from either HSV-1-infected (■) or uninfected (□) BHK cells, and HSV-1-infected ribonucleotide reductase activity was assayed as described in Fig. 1 legend. The assay was modified to measure mammalian enzyme activity and contained 100 mM HEPES pH 7.6, 2.7 mM ATP, 2.7 mM magnesium acetate, 10 mM DTT, 55 µM CDP and 1 µCi ^3H -CDP (10–30 Ci mmol $^{-1}$). The enzyme activity is expressed as a percentage of that found in the absence of oligopeptide. The 100% values correspond to 51,806 and 17,064 c.p.m. of ^3H -dCDP formed by the infected and uninfected cell extracts, respectively.

of the small subunit involved in interacting with the large subunit. We suspected that the synthetic peptide might compete for a site on the large subunit and displace the small subunit.

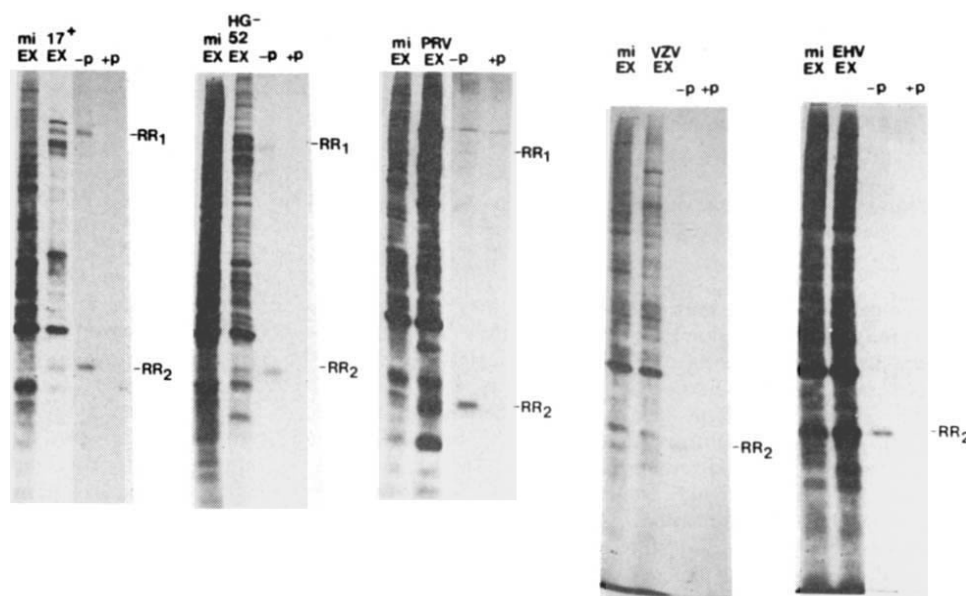
Ribonucleotide reductase activity in extracts of HSV-1-infected and mock-infected cells was assayed in the presence of various concentrations of the synthetic oligopeptide YGAVVNDL (peptide 1). The results show that peptide 1 inhibits virus-specific activity (Fig. 1). Peptide 2 (NH $_2$ -Thr-Ser-Arg-Leu-Ser-Asp-Pro-Asn-Ser-Ser-Ala-Tyr-COOH, TSRLSDPNSSAY), which corresponds to the amino-terminal sequence of the 10K protein product of the HSV-1 gene US9 (ref. 10), was included in the experiment as a control and did not inhibit activity.

A comparison of the predicted amino-acid sequences of ribonucleotide reductases from HSV-1, HSV-2, EBV, *Escherichia coli*, clam and sea urchin^{11–15} and varicella zoster virus (VZV)¹⁶ shows that the tyrosine residue at position 9 from the carboxy-terminal end of the herpesvirus RR $_2$ is conserved in all species¹⁷; this led us to speculate that this tyrosine was particularly important for enzyme function. We therefore tested two further oligopeptides, one corresponding to the carboxy-terminal nonapeptide NH $_2$ -Tyr-Ala-Gly-Ala-Val-Val-Asn-Asp-Leu-COOH (YGAVVNDL, peptide 3) and differing from peptide 1 by the inclusion of an alanine residue between the tyrosine and glycine residues, and the other a tridecapeptide NH $_2$ -Ser-His-Thr-Asn-Phe-Phe-Glu-Cys-Arg-Ser-Thr-Ser-Tyr-COOH (HTNFFECRS-TSY, peptide 4), which corresponds to the conserved tyrosine residue and the adjoining 12 amino acids on the amino-terminal side. Figure 1 shows that peptide 3 is a more effective inhibitor than peptide 1 whereas peptide 4 is at best only marginally inhibitory. In this experiment the concentration of peptide giving 50% inhibition of enzyme activity was 4.5 µg per assay for peptide 3 and 21 µg per assay for peptide 1, which shows that peptide 3 is approximately five times more inhibitory than peptide 1.

To test whether the inhibition of ribonucleotide reductase activity by peptide 3 was specific for the virus-induced enzyme, we investigated the effect of the peptide on the viral and cellular enzymes. Figure 2 shows that the inhibition is specific for the virus-induced enzyme and has little effect on the cellular enzyme.

The highly conserved carboxy-terminal amino-acid sequence of HSV-1 and HSV-2 (refs 12, 13), EBV¹⁵ and VZV¹⁶ led us to test whether the antigenic domain(s) recognized by the anti-peptide 1 serum described above is also conserved in other herpesviruses. Extracts from cells infected with HSV-1, HSV-2, VZV, PRV and EHV-1 were made and immunoprecipitations performed as described elsewhere⁹. In those experiments no immunoprecipitation of any cellular proteins was observed. Figure 3 shows that RR $_2$ is specifically immunoprecipitated from extracts of cells infected with any of these herpesviruses and that RR $_1$ is co-precipitated from cells infected with HSV-1, HSV-2 and PRV but not VZV and EHV-1. This result suggests that PRV, like HSV-1 and HSV-2, has two types of interacting ribonucleotide reductase subunits and that their site of interaction is likely to be a common target for the inhibitory oligopep-

Fig. 3 Immunoprecipitation of polypeptides from extracts of cells infected with herpesviruses HSV-1 (strain 17 syn $^+$), HSV-2 (strain HG52), PRV, VZV and EHV-1, using IgG purified from an antiserum (14119) raised against the carboxy-terminal seven amino acids of the small ribonucleotide reductase subunit (RR $_2$) of HSV-1 and HSV-2. ^{35}S -methionine-labelled extracts of BHK cells infected with HSV-1, HSV-2 and PRV were prepared as described elsewhere⁹. Rabbit kidney (RK) cells were infected with EHV-1 in a similar manner. HFL cells were infected with VZV by co-culturing confluent monolayers with VZV-infected HFL cells. Immunoprecipitations were carried out as described elsewhere⁹ in extraction buffer containing 0.5% SDS. IgG was purified from antiserum 14119 by ion-exchange chromatography and used in immunoprecipitations with (+p) and without (–p) 100 µg of the synthetic peptide 1, against which the antiserum was raised⁹. Lanes designated mi EX, 17 $^+$ EX, HG52 EX, PRV EX, VZV EX and EHV EX show ^{35}S -methionine-labelled polypeptide profiles for mock-infected, HSV-1 (strain 17 syn $^+$), HSV-2 (strain HG52), PRV, VZV and EHV-infected cell extracts, respectively. RR $_1$ and RR $_2$ are, respectively, the large and small herpesvirus ribonucleotide reductase subunits.



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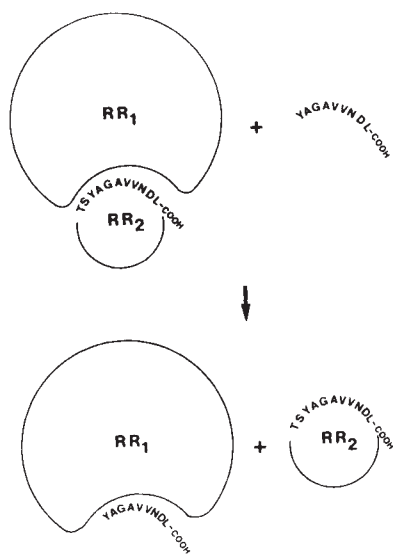


Fig. 4 Possible mechanism for inhibition of the herpes virus ribonucleotide reductase by the synthetic peptide 3.

tides. For EHV-1 and VZV the situation is less clear—different conditions may well be necessary to co-precipitate RR₁ with RR₂ from cells infected with these viruses.

Figure 4 presents a model to explain the inhibition of enzyme activity based on evidence indicating that the two subunits interact via the carboxy terminus of RR₂ (ref. 9). The model proposes that the inhibitory nonapeptide prevents the association of the two subunits by competing for the subunit binding site on RR₁, displacing RR₂. The model is not intended to imply that only one large and one small subunit combine to form the active enzyme, or that the carboxy-terminal region is the only site involved in the interaction between the small and large subunits.

The potential therapeutic implications of specific inhibition of the subunit interaction are being investigated. Exposure of infected cell monolayers to various concentrations of the nonapeptide (peptide 3) did not reduce the yield of infectious virus; this is probably because the peptide is too large to enter the cell unaided. Present efforts are directed towards identification of smaller peptides which specifically inhibit ribonucleotide reductase activity both *in vitro* and *in vivo*.

We thank Mrs M. Murphy, Mrs A. Orr and Mr G. Hope for technical assistance; Dr J. B. Clements, Dr A. J. Davison and Dr J. McLauchlan for helpful discussions; and Ms A. Cullinane for providing the stock of EHV. We also thank Miss F. Conway and Mrs A. Simpson for typing the manuscript.

Received 23 December 1985; accepted 18 March 1986.

Specific inhibition of herpesvirus ribonucleotide reductase by a nonapeptide derived from the carboxy terminus of subunit 2

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Ribonucleotide reductase, an essential enzyme for the synthesis of deoxyribonucleotides, is formed by the association of two non-identical subunits in almost all prokaryotic and eukaryotic cells¹. The same model probably holds for the herpes simplex virus (HSV)-encoded ribonucleotide reductase²⁻⁶; two polypeptides of relative molecular mass 136,000 (136K; H1) and 40K (H2) (referred to elsewhere as RR₁ and RR₂; see for example, Dutia *et al.*²⁶) have been associated with the viral enzyme by both genetic^{7,8} and immunological⁹⁻¹¹ studies. Furthermore, DNA sequence analyses have shown significant stretches of amino-acid homology between these viral polypeptides and those of, respectively, subunit 1 (ref. 12) and subunit 2 (ref. 13) of the *Escherichia coli* and mammalian enzymes. To assess the involvement of the 40K polypeptide in reductase activity, we synthesized a nonapeptide corresponding to the sequence of its carboxy terminus with the intention of raising neutralizing antibodies specific for the viral activity (E.A.C. *et al.*, in preparation). We report here the unexpected finding that the nonapeptide itself specifically inhibits the HSV ribonucleotide reductase activity in a reversible, non-competitive manner, and we suggest that it does this by impairment of the correct association of the two subunits. This phenomenon emphasizes the potential usefulness of synthetic peptides in probing critical sites involved in macromolecular interactions.

The nonapeptide NH₂-Tyr-Ala-Gly-Ala-Val-Val-Asn-Asp-Leu-COOH, corresponding to the carboxy-terminal nine amino acids of the HSV type 1 (HSV-1) 40K and HSV-2 38K polypeptides (as predicted from the DNA sequences^{14,15}), was synthesized by the solid-phase technique of Merrifield¹⁶ and its primary structure verified by quantitative amino-acid analysis after acid hydrolysis and digestion with leucine aminopeptidase. The preparation was 99% pure as determined by HPLC. HSV-1 or HSV-2 ribonucleotide reductase was partially purified from quiescent BHK-21/C13 cells infected respectively with strain F and strain HG-52 at 20 plaque-forming units (PFU) per cell, whereas the cellular enzyme was obtained from exponentially growing BHK-21/C13 cells or hydroxyurea-resistant 96-V-2(600) cells. The latter cells exhibit high ribonucleotide reductase specific activity owing to overproduction of the two enzyme subunits (ref. 17 and our unpublished observations for subunit 1).

Figure 1 shows the effect of the synthetic peptide on the HSV-1 enzyme: 50% inhibition was observed with 22 µM nonapeptide and total inhibition was obtained with a concentration of 400 µM. The curve obtained for the HSV-2 enzyme was almost identical (data not shown). The cellular enzyme extracted from 96-V-2(600) (Fig. 1) or BHK-21/C13 cells (data not shown) was completely insensitive to the presence of the peptide in the assay up to a concentration of 1 mM. To show that this specific effect was due to the nonapeptide rather than attributable to some trace contaminants, we used an anti-nonapeptide rabbit serum with specific neutralizing activity against the HSV ribonucleotide reductase activity (E.A.C. *et al.*, in preparation). Purified IgG (5 µg, which gave 60% neutralization in the absence of peptide) was pre-adsorbed with increasing concentrations of nonapeptide

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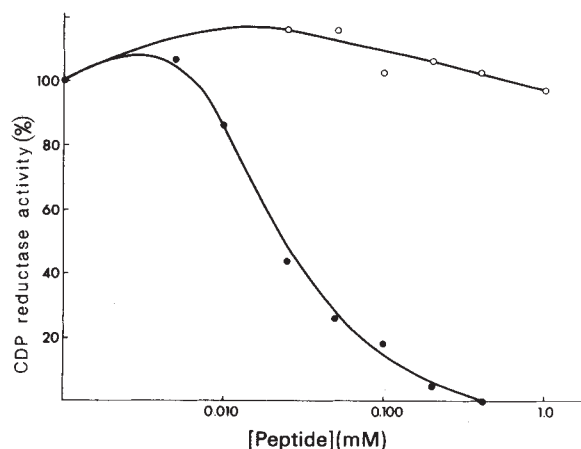


Fig. 1 Effect of the synthetic nonapeptide on viral (●) and cellular (○) ribonucleotide reductase activities. One unit (U) of ribonucleotide reductase activity is defined as the amount of enzyme generating 1 nmol of dCDP per h under the standard assay conditions. The results are expressed as a percentage of the activity obtained in the controls without nonapeptide.

Methods. Viral and cellular ribonucleotide reductases were, respectively, partially purified from HSV-1 (strain F)-infected confluent BHK-21/C13 cells and exponentially growing 96-V-2(600) cells by precipitation of the cell extract with ammonium sulphate as described elsewhere⁶. Aliquots of the viral (50 µg; specific activity 30 U mg⁻¹) or cellular (40 µg; specific activity 34 U mg⁻¹) enzyme preparation were mixed with increasing concentrations of nonapeptide, and ribonucleotide reductase activity was assayed by monitoring the reduction of CDP as described previously⁶. Briefly, the standard reaction mixture, in a final volume of 60 µl, contained: 50 mM HEPES pH 7.8, 4 mM MgCl₂, 4 mM ATP, 4 mM NaF, 6 mM DTT, 54 µM CDP and 0.25 µCi ³H-CDP. For the viral activity, MgCl₂ and ATP (which are non-essential for this activity) were usually omitted and 20 mM DTT was used instead. These changes did not affect the inhibition by the nonapeptide. After 30 min of incubation at 37 °C, the reaction was stopped by immersing the tube in boiling water for 4 min and the precipitate removed by centrifugation. Nucleotides in the supernatant were converted to nucleosides by enzymatic hydrolysis. The deoxyribonucleosides were subsequently separated from the ribonucleosides by ascending polyethyleneimine-cellulose chromatography.

and preincubated with 50 µg of viral enzyme before the ribonucleotide reductase assay. At low nonapeptide concentrations, the ability of the IgG to neutralize the enzyme activity was progressively eliminated until equivalence was reached at 10 µM (see Fig. 2). Subsequently, the activity was inhibited but the curve was displaced to the right relative to that in Fig. 1, as expected if the nonapeptide is responsible for the inhibition.

As further controls for the specificity of the inhibition, we also examined the effect of the nonapeptide on other HSV-encoded enzymatic activities involved in DNA metabolism: thymidine kinase, DNA polymerase and DNase. These viral enzymes were specifically measured respectively by phosphorylation of ³H-bromodeoxycytidine¹⁸, ³H-dGTP incorporation in activated DNA in the presence of 150 mM ammonium sulphate¹⁹, and solubilization of ³H-thymidine from *in vivo* labelled DNA assayed at pH 9.0 (ref. 20). None of these enzymatic activities was affected by the presence of 1 mM of the nonapeptide (data not shown), indicating that the action of the inhibitor was specific to the HSV ribonucleotide reductase activity.

We next investigated the mechanism of action of this inhibitor. As HSV ribonucleotide reductase activity could be completely restored when the nonapeptide was removed by dialysis from a preparation of enzyme preincubated with 1 mM nonapeptide, we concluded that the inhibition was reversible. Preincubation of the peptide with the viral enzyme did not change the degree of inhibition observed at given peptide concentrations. Measurement of the initial rate of product formation in the presence of

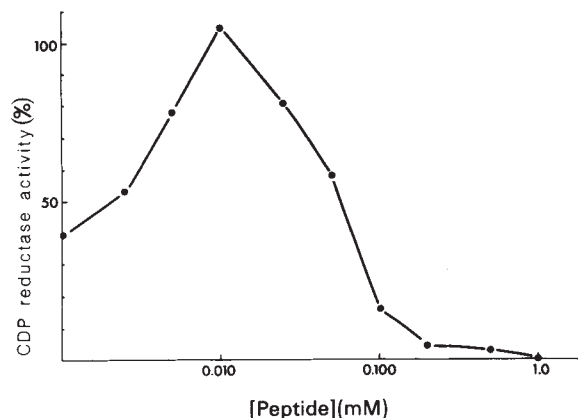


Fig. 2 Effect of the nonapeptide on the neutralizing activity of an anti-nonapeptide antiserum. The anti-nonapeptide antiserum (P9) was obtained by immunizing a rabbit with the nonapeptide conjugated to methylated bovine serum albumin, as described elsewhere²⁵. IgGs were purified from the P9 serum by adsorption to a column of protein A-Sepharose CL-4B (Pharmacia), followed by elution with 0.1 M glycine pH 2.5. After incubation of 5 µg of P9 IgGs with increasing concentrations of nonapeptide for 30 min at 20 °C, 50 µg of partially purified HSV-1 ribonucleotide reductase (specific activity 30 U mg⁻¹) was added to each sample. After a second incubation of 30 min at 20 °C, ribonucleotide reductase activity was assayed as outlined in Fig. 1 legend.

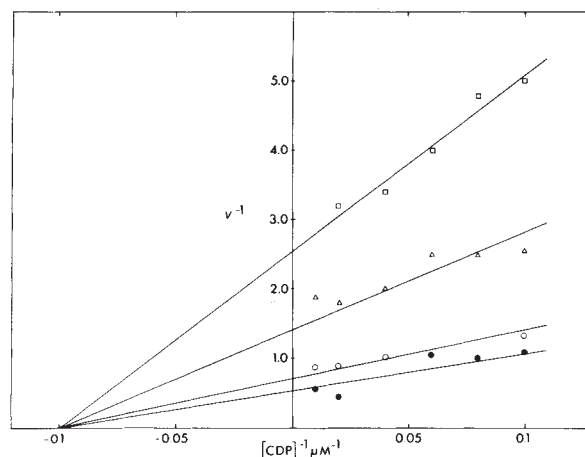


Fig. 3 Double-reciprocal plot of velocity (v) versus CDP concentrations at different nonapeptide concentrations. Aliquots of partially purified HSV-1 enzyme preparation were mixed with the following nonapeptide concentrations: 0 µM (●), 15 µM (○), 25 µM (△), 50 µM (□), and assayed using the standard reaction mixture as described in Fig. 1 legend, except that CDP was added at the appropriate concentrations.

inhibitor revealed that the change in the velocity (v) of the reaction was very rapid, being already established after 2 min. These results suggested that the peptide did not need any modification by a factor present in the extract to become active. The nature of the inhibition was determined by varying the CDP concentration at several fixed concentrations of nonapeptide. By means of a double reciprocal plot, we demonstrated that the nonapeptide behaved as a non-competitive inhibitor with respect to CDP (Fig. 3). The K_i , calculated by re-plotting $1/v$ against the inhibitor concentration, gave a value of 15 µM. By using the same type of analysis, we determined that this inhibitor also does not compete with the reducing agent dithiothreitol (DTT).

To explain the unexpected inhibitory activity of the nonapeptide, we hypothesize that the carboxy terminus of the presumptive subunit 2 (H2) of the HSV ribonucleotide reductase

is directly involved in binding with subunit 1 (H1). Thus, the carboxy-terminal nonapeptide would compete with H2 for some critical binding site and effectively prevent the formation of the active holoenzyme; this notion is supported by the observed specificity of the inhibition for the viral enzyme, as the nonapeptide does not share significant amino-acid homology with subunit 2 of the cellular enzyme¹³ and thus presumably does not interact with the cellular subunit 1. Our findings that the inhibition is reversible and non-competitive with respect to the substrate and the reducing agent are also in agreement with this hypothesis. However, at present we cannot exclude other possibilities such as the inhibition by the nonapeptide of some essential interaction between viral subunit 2 and other cellular protein(s) or constituent(s). These hypotheses should be testable by crosslinking studies using radiolabelled nonapeptide.

Here we report that a synthetic peptide derived from the amino-acid sequence of an enzyme behaves as a specific inhibitor of the enzyme, adding another area of research in which synthetic peptides have proved useful. Synthetic peptides have been used largely to examine the interactions between proteins and receptors²¹⁻²⁴. We predict that the use of such synthetic peptides, derived from amino-acid sequences implicated in the association of proteins with receptors, membranes, DNA, RNA or enzyme subunits, will be of prime importance in elucidating macromolecular interactions. Moreover, it is tempting to speculate that synthetic peptides could have potential in antiviral chemotherapy. In this respect, we are testing the effect of our nonapeptide on replication of HSV.

We thank Claire Guilbault for technical assistance and Lucille Beaudet for the assays of HSV thymidine kinase, DNA poly-

merase and DNase. We also thank W. H. Lewis for his gift of the 96-V-2(600) cell line and V. Bibor-Hardy and W. E. C. Bradley for critical reading of the manuscript. This work was supported by grants from NCI (Canada) to Y.L. and from MRC (Canada) to P.B. E.A.C. is the recipient of a studentship from La Société de Recherche sur le Cancer and P.G. of a postdoctoral fellowship from FRSQ (Québec).

Received 23 January; accepted 10 March 1986.

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Re-routing of a secretory protein by fusion with human growth hormone sequences

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Cells with electron-dense secretory vesicles use them to store only specialized secretory products such as peptide hormones; other types of secreted proteins are externalized by an alternative, constitutive route¹. One possible mechanism for such segregation is that proteins destined for dense secretory vesicles contain unique 'sorting domains'² that allow for selective targeting. Here, we set out to determine whether a constitutively secreted protein could be diverted to the dense secretory vesicles by attachment to a peptide hormone sequence. We made use of the ability of the mouse pituitary tumour cell line, AtT-20, to correctly sort exogenous secretory proteins introduced into them by DNA transfection³. We constructed a plasmid encoding a hybrid protein in which a constitutively secreted viral protein was fused to the carboxy terminus of human growth hormone (hGH). Cells expressing the hybrid protein were found to target it to dense secretory vesicles with an efficiency close to that observed for the parental hGH. These results support the hypothesis that sorting domains on peptide hormones direct their packaging into dense secretory vesicles. The results also suggest that proteins secreted by the constitutive pathway either do not contain any sorting domain, or their sorting signals can be overridden by those which direct peptide hormones.

Generation of fusion proteins is a useful means of studying the targeting of nuclear, mitochondrial, vacuolar and membrane proteins to their correct destinations⁴⁻⁹. To construct a hybrid

between a constitutively secreted protein and a protein destined for dense secretory vesicles (that is, destined for the regulated pathway), we fused the hGH sequence to the truncated vesicular stomatitis virus (VSV) G sequence (Fig. 1a). The truncated G protein (designated TG) contains an intact 432-amino-acid luminal region of the G protein but lacks the entire membrane-spanning and cytoplasmic domains (79 amino acids from the carboxy terminus)¹⁰. When transfected separately into AtT-20 cells, hGH was found to be transported to the regulated pathway while TG was exported efficiently by the constitutive pathway³. Figure 1b illustrates the strategy for constructing a precise in-frame fusion between the two coding sequences. An hGH complementary DNA including 35 bases of the 5'-untranslated sequence¹¹ was first inserted in the appropriate orientation between the Rous sarcoma virus (RSV) long terminal repeat (LTR) promoter and the TG coding sequences in an expression vector (step 1 in Fig. 1b). A fragment containing the hGH-TG sequences was subcloned into an M13 vector, and a 200-base pair (bp) sequence at the junction of hGH and TG was deleted by oligonucleotide-directed mutagenesis (steps 2, 3 in Fig. 1b). As shown in Fig. 1a, the resulting sequences encode a hybrid protein in which the last amino acid of hGH is joined directly to the first amino acid of the mature TG protein, which lacks its signal peptide. We found that ~2% of the M13 phage plaques had incorporated the desired mutation. To ensure that no other mutations had been introduced during the mutagenesis procedure, we sequenced the entire insert, including regions outside the mutated area, before cloning it back into the RSV-LTR expression vector (step 4 in Fig. 1b).

Figure 2 shows immunoprecipitation analyses of cellular and secreted proteins from an AtT-20 cell line that had been stably transformed with the chimaeric plasmid described above. As neither hGH nor TG is normally processed from larger precursor propeptides, it was predicted that the fusion protein would be a single polypeptide with a relative molecular mass (M_r) equal to the sum of those of hGH (22,000; SSK) and TG (54-56K). The transformed cells synthesized a protein with an apparent

Fig. 1 *a*, Structure of a hGH-TG hybrid created by oligonucleotide-directed mutagenesis. A 30-mer mutagenic primer was used to delete the hGH-TG junctional region, including the stop codon and the 3'-untranslated sequences of hGH, and the 5'-untranslated sequences and the 16-amino-acid signal peptide of the truncated G protein. Half of the primer is complementary to the 15-base sequence immediately downstream from the signal peptide of TG; the other half is complementary to the last 15 bases of the mature hGH coding sequences. *b*, Construction of an expression vector containing the hGH-TG hybrid. The full-length hGH cDNA, carried on a *Hind*III fragment (chGH800/pBR322; ref. 11), was inserted in the *Hind*III site of pRSV-TG, the plasmid vector used previously to express a truncated VSV G protein in AtT-20 cells³ (step 1). A 3.0-kilobase (kb) *Bam*HI fragment spanning the entire hGH and truncated G sequences was then subcloned into M13mp11 (step 2). The fusion junction was modified by site-directed mutagenesis using the single-stranded M13 DNA as a template (step 3; see *a*). An 814-base pair (bp) fragment encompassing the modified fusion junction was then cloned back into the expression vector pRSV-hGH-TG as a *Nar*I/*Nco*I fragment (step 4). ▨, RSV LTR; ■, hGH sequences; □, TG sequences; ▩, simian virus 40 splice junction and polyadenylation site; —, pBR322 sequences; —, mutagenic primer.

Methods. pRSV-TG (100 ng) was digested with *Hind*III, treated with phosphatase and then ligated to 100 ng of an 803-bp *Hind*III fragment which had been excised from chGH800 and purified on agarose gels. Plasmids with the hGH sequences inserted in the same orientation as TG were isolated and digested with *Bam*HI. The 3.0-kb fragment was purified on agarose gels, ligated to *Bam*HI-digested, phosphatase-treated M13mp11 vector, and used to transform *Escherichia coli* JM101. Single-stranded phage DNA was prepared and sequenced by the dideoxy method¹⁸ to ensure the correct orientation of the insert. One pmol of the single-stranded template was annealed simultaneously to 20 pmol of 5' phosphorylated mutagenic primer and 20 pmol of a second, 15-base primer. The latter anneals to sequences 180 bp away from the mutagenic primer, in the TG coding region. After *in vitro* synthesis in the presence of DNA polymerase large fragment (Klenow) and *T*₄ ligase, the reaction mixture was used to transform *E. coli* JM101. Phage plaques carrying the desired mutation were identified by hybridization to [³²P]-labelled mutagenic primer, followed by washing under conditions of appropriate stringency. DNA from the purified plaque was sequenced by dideoxy sequencing to confirm the mutation. The *Nar*I/*Nco*I fragment was then excised from the M13 vector, gel-purified, and ligated to pRSV-GH-TG, which had been digested with *Nco*I and *Nar*I and treated with phosphatase.

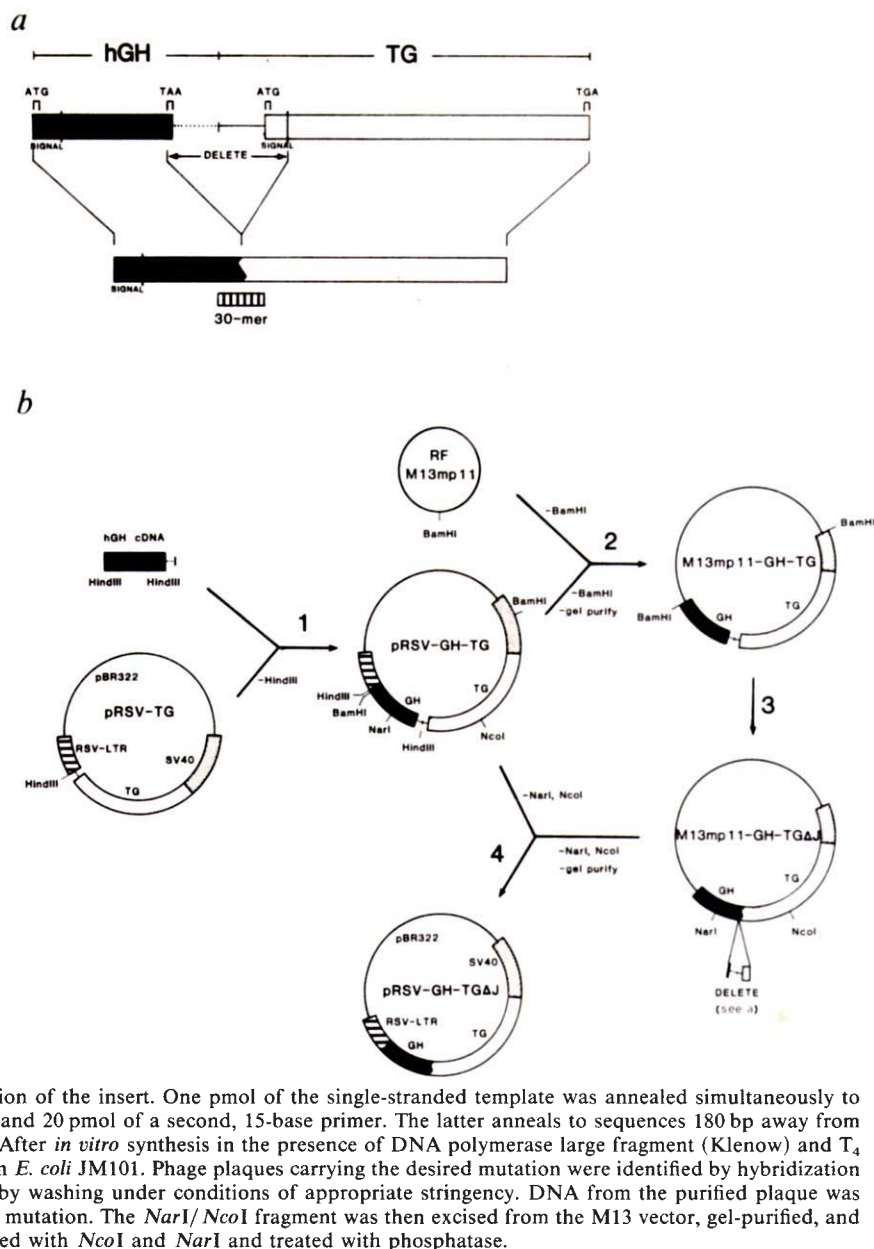
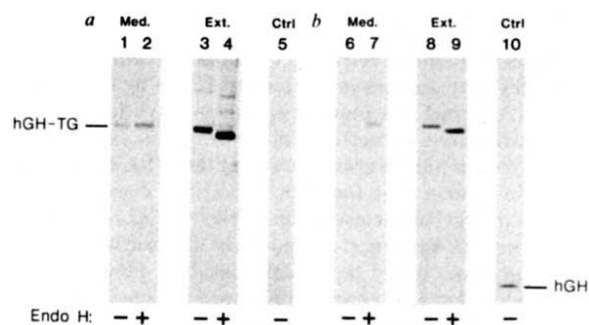


Fig. 2 Transformed AtT-20 cells synthesize and secrete a glycosylated hGH-TG hybrid protein. An AtT-20 cell line stably transformed with the plasmid pRSV-GH-TGΔJ was radiolabelled with ³⁵S-methionine for 13 h. Cellular and secreted proteins were then analysed by immunoprecipitation with anti-VSV (*a*) or anti-hGH (*b*) antisera, followed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE). Lanes 1, 2, 6 and 7 are autoradiograms of immunoprecipitates from samples of medium, and lanes 3, 4, 8 and 9 are from cell extracts. As a control, samples of medium from cells expressing hGH protein not fused to TG were analysed in parallel (lanes 5, 10). Samples in lanes 2, 4, 7 and 9 were digested with Endo H before electrophoresis.

Methods. A 10-cm dish of AtT-20 cells was transfected with 120 μg of pRSV-GH-TGΔJ and 24 μg of pSV2-neo by the calcium phosphate procedure³. Clones surviving the G-418 selection were picked and screened by hGH radioimmunoassay (Hybritech). Cells from a positive clone, I17, were labelled, and medium and extract samples were prepared and analysed according to published procedures³.



M_r of ~72–75K that could be precipitated by both anti-VSV (Fig. 2, lane 3) and anti-hGH (Fig. 2, lane 8) antisera. The specificity of the immunoprecipitation reaction was demonstrated by the fact that authentic hGH could be precipitated only by the anti-hGH (Fig. 2, lane 10), not the anti-VSV (lane 5) antiserum. The fusion polypeptide was secreted into the culture medium by the transformed cells (Fig. 2, lanes 1, 6), and the secreted form exhibited similar immunoreactivity with both antisera. The fusion protein could also be detected by a hGH radioimmunoassay (Hybritech, La Jolla, California) (data not shown); by this assay, the rate of synthesis of the hybrid protein was estimated to be 0.3 pmol per h per mg cell protein. As this is much less than the level of the endogenous hormone, adrenocorticotrophic hormone (ACTH; 18 pmol per h per mg cell protein), it is unlikely to perturb the sorting machinery.

The glycosylation pattern of the fusion protein suggests that it traverses the normal secretory compartments before secretion. VSV G has two *N*-linked oligosaccharide addition sites whereas hGH is not glycosylated. The fusion protein acquires post-translational glycosylation similar to that of the truncated G protein. The predominant intracellular form of the fusion protein bears oligosaccharides of the high mannose type and is sensitive to endoglycosidase H (Endo H) digestion (Fig. 2, lanes 3, 4 and 8, 9). A minor band with a slightly slower mobility, not apparent in the exposure of Fig. 2, was also detected in the cell extracts, especially after 1–2 h of chase (not shown); this form bears oligosaccharides of the complex type and is resistant to Endo H digestion. In contrast, the secreted fusion protein is exclusively Endo H-resistant (Fig. 2, lanes 1, 2 and 6, 7). Almost identical patterns were also found for ACTH (not shown).

The above results suggest that the conformation of the two parent proteins has not been drastically altered in the hybrid protein. First, the hGH moiety of the hybrid preserved sufficient antigenicity for measurement by radioimmunoassay and immunoprecipitation (note that a monoclonal antibody and two different polyclonal antibodies were used). Similarly, the TG portion retained sufficient antigenicity to be recognized by an anti-VSV-specific antiserum. Furthermore, the glycosylation sites on TG were still available.

To determine whether the hGH sequences could divert TG to the dense secretory vesicles, we examined the effects of secretagogues on the secretion of the hybrid protein. As we have shown previously, secretagogues stimulate the secretion of only those proteins that are packaged in dense secretory vesicles¹², thereby providing a simple test for the secretory pathway taken by the hybrid protein. Cells were labelled with ³⁵S-methionine for 16 h and then chased for 6 h to reduce the background contributed by constitutive secretion³. We then determined the effect of the secretagogue 8-bromocyclic AMP (8-Br-cAMP) on the rate of secretion. We have demonstrated previously that secretagogue-stimulated cells show enhanced secretion of hGH but not of TG, indicating that only the former is packaged into the regulated secretory vesicles. Those results were confirmed here (Fig. 3b). Under the same conditions, secretion of the hGH-TG fusion protein was clearly stimulated (Fig. 3a (lanes 3, 4) and b). To quantitate the extent to which the hybrid protein is sorted into the regulated pathway, we calculated the increase in the amount of secretion induced by 8-Br-cAMP and normalized it to the steady-state rate of secretion. The 'sorting indices' obtained by such quantitation (described in detail in ref. 3) indicate that the hGH-TG hybrid enters the regulated secretory vesicles with ~50% the efficiency of hGH (0.43 for hGH compared with 0.22 for hGH-TG; see legend to Fig. 3b). This extent of sorting is at least comparable to, if not better than, that of the endogenous hormone ACTH (0.14; see Fig. 3b). Thus, because of the attachment of hGH sequences, the truncated G protein has acquired the ability to be packaged into regulated secretory vesicles.

Conceivably, sorting of proteins into the two pathways could be achieved by means of sorting domains present on either the

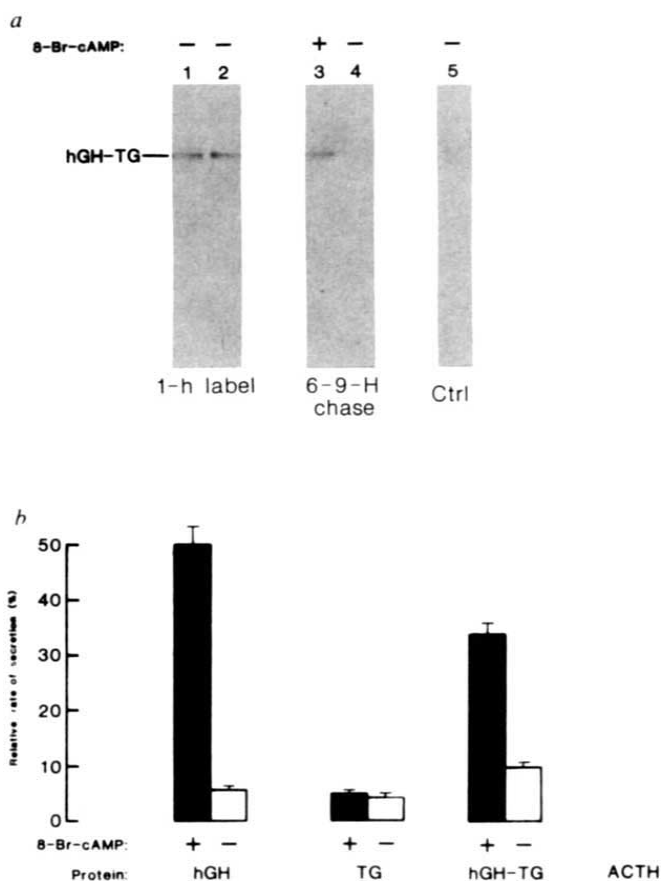


Fig. 3 The hGH-TG hybrid protein is secreted by a secretagogue-dependent, regulated pathway. *a*, Lanes 1 and 2, secretion of ³⁵S-methionine-labelled hybrid proteins from two identical dishes before stimulation. The amount represents total secretion during 1 h of continuous labelling. Lanes 3 and 4, secretion from stimulated and unstimulated cultures, respectively, during a 3-h chase period. Lane 5, control medium collected from untransfected AtT-20 cells. *b*, Comparison of the effects of the secretagogue 8-Br-cAMP on the secretion of hGH, TG and the hGH-TG hybrid protein. For each protein, the rate of secretion in the presence (+) or absence (-) of 5 mM 8-Br-cAMP was measured after cells had been radiolabelled for 16 h and chased for 6 h as in lanes 3 and 4 of *a*. Radioactivity associated with each protein was quantitated by scanning the autoradiograms, and was corrected for the duration of stimulation. Values plotted have been normalized to the rate of secretion measured immediately before the chase (lanes 1 and 2 in *a*). Sorting indices were calculated as described previously³.

Methods. Two 10-cm dishes of I17 cells were each incubated with 0.5 mCi ³⁵S-methionine for 15 h to approach steady-state labelling. For normalization, proteins secreted into the medium during the ensuing hour were collected in the continuing presence of the radiolabel (lanes 1 and 2 in *a*). To test the effect of 8-Br-cAMP on secretion, the labelled cells were first chased for 6 h. During the next 3-h chase, one dish was stimulated with 5 mM 8-Br-cAMP and the other was not. Samples of medium were collected and immunoprecipitated with excess anti-VSV antiserum (lanes 3 and 4 in *a*). For controls, cells synthesizing unfused hGH or TG protein³ were analysed in parallel using anti-hGH or anti-TG antiserum, respectively. Immunoprecipitation was carried out with the following modifications. Samples were incubated with excess antiserum and precipitated with *Staphylococcus aureus* cells, then the immunoprecipitates were eluted from the cells in 1% SDS at 37 °C for 20 min. The supernatant was diluted to 0.2% SDS and additional antiserum added. After further precipitation with *S. aureus* cells, the immunoprecipitates were eluted from the cells by boiling in Laemmli gel sample buffer. Under these conditions, the specific binding was not significantly altered but the background was drastically reduced¹⁷.

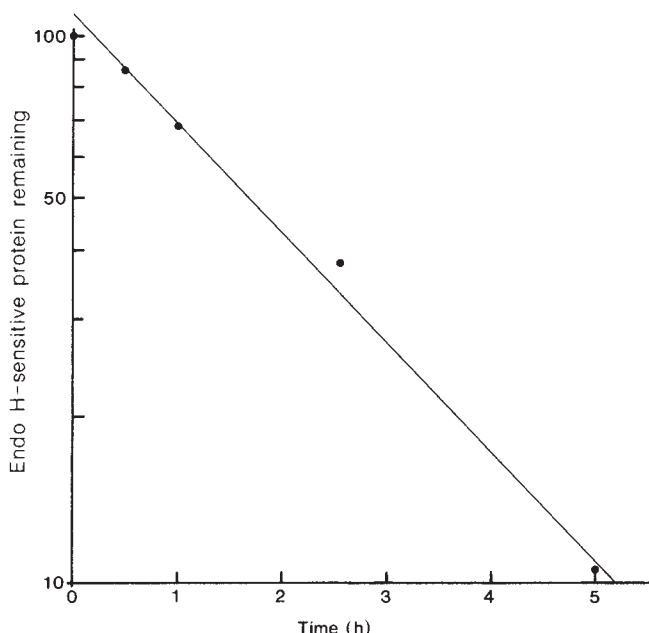


Fig. 4 hGH-TG hybrid protein exits from the RER at a rate characteristic of the TG protein. Cells were pulse-labelled with ^{35}S -methionine and chased for the times indicated. At each time point, the fraction of hybrid protein remaining sensitive to Endo H digestion was calculated from the total hybrid protein recovered in the cell extract and in the medium. Linear regression analysis shows a $t_{0.5}$ of 100 min for the conversion of Endo H-sensitive to Endo H-resistant forms.

Methods. Identical wells of I17 cells were pulse-labelled with ^{35}S -methionine for 10 min, then chased for 30 min, 1 h, 2.5 h and 5 h. At each time point, medium was collected and cells were extracted with detergents³. Forty per cent of the extract samples and 100% of the medium samples were immunoprecipitated with an anti-VSV antiserum, digested with Endo H, and analysed by 15% SDS-PAGE. The radioactivity associated with each form was quantitated by scanning the bands and correcting for the amount analysed.

constitutive or the regulated secretory proteins. The other class of proteins would then be transported to the second pathway by a nonspecific, bulk-flow process. Alternatively, both classes may contain sorting signals. Our data support the idea that sorting is effected by active signals on proteins destined for the regulated pathway. The constitutively secreted TG protein may lack any sorting signal, or its sorting domain may be subordinate to those of the peptide hormones. The suggestion that sorting domains are not a mandatory requirement for proteins to exit via the constitutive pathway is consistent with several earlier observations. Under conditions where intracellular targeting is perturbed by drug treatment¹³, genetic disorder¹⁴ or overproduction of the transported proteins (T. H. Stevens, G. Payne and R. Schekman, in preparation), the mis-localized proteins from the dense secretory vesicles, lysosomes or vacuoles do not accumulate within the cells, but are secreted continuously into the medium. The constitutive pathway thus appears to be a default route for any soluble protein that cannot find another destination. Therefore, the effective diversion of TG by hGH sequences probably reflects a lack of sorting signals on the VSVG fragment.

While the hGH domain appears to dominate the post-Golgi sorting of the fusion protein, its rate of movement out of the rough endoplasmic reticulum (RER) resembles more closely that of the truncated VSV G protein. In all cell lines examined previously, hGH is transported rapidly out of the RER, the half time ($t_{0.5}$) of RER export in transfected AtT-20 cells being less than 20 min (J. Rose, personal communication, and H.-P.H.M., data not shown). In contrast, TG leaves the RER much more slowly^{3,10}, with $t_{0.5}$, as measured by the acquisition of Endo H-

resistant terminal glycosylation, being 90–100 min. Using similar criteria, we found that conversion of the hGH-TG hybrid from the Endo H-sensitive to the Endo H-resistant forms occurred at a rate comparable to that of TG (Fig. 4): 50% conversion occurred at ~100 min after the chase. Thus the slow movement of the hybrid protein from the RER appears to predominate over the fast movement.

Cellular carriers or receptors have been proposed to explain the different rates at which secreted proteins move from the RER to the Golgi apparatus^{15,16}. If such carriers recognize features of newly synthesized proteins, the fusion protein strategy should be equally suited to identifying such features. It is noteworthy, therefore, that the rate at which the fusion protein exits from the RER is determined by a different region from that which determines its sorting into secretory vesicles. If export from the RER were indeed carrier-mediated, it would involve a receptor different from that which effects targeting to the secretory vesicles. An alternative, perhaps more attractive, hypothesis consistent with our observations is that movement out of the RER does not involve specific carriers. In this case, export of TG (and therefore of its fusion product) is retarded simply as a result of its delayed folding as an abnormal secretory protein in the RER.

This work was supported by NIH grant AM 33937 (to R.B.K.), and by Juvenile Diabetes Career Development Award 284087 and NIH grant GM 35239-01 (to H.-P.H.M.). We thank Dr Steve Gardell for assistance in the mutagenesis techniques; Dr Kevin Moore, Dr Peter Walter and Ms Sharon Powell for critical reading of the manuscript; and Mr David Quinn for the graphic work.

Received 6 January; accepted 14 March 1986.

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Expression of biologically active viral satellite RNA from the nuclear genome of transformed plants

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The particles of cultures of cucumber mosaic virus (CMV) contain the three genomic species of single-stranded RNA and a sub-genomic species that acts as messenger RNA for CMV particle protein. Some cultures also contain a single-stranded linear RNA molecule that is typically ~335 nucleotides long^{1,2}. This extra molecule, termed satellite RNA, does not share appreciable nucleotide sequence with CMV genomic RNA³ but replicates only in

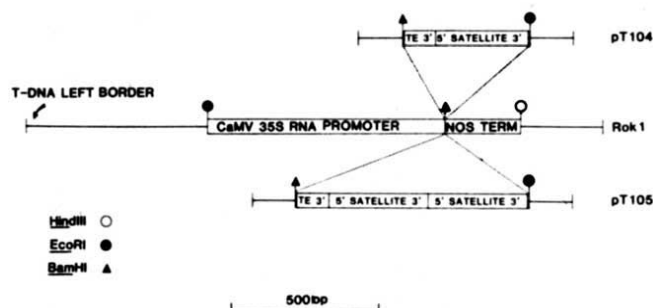


Fig. 1 Structure of the CMV satellite cDNA molecules in the binary Ti plasmid expression vectors. The cloning and nucleotide sequence of the CMV satellite RNA will be presented elsewhere. Briefly, at the 5' end of the 104 construction there are 51 nucleotides from the polylinker of plasmid pT7-2 extending from the transcriptional start point and including polylinker sequences of pT7-2 and other vectors used in the construction of pT104. On the 3' end of this, as shown in the diagram, there are 108 nucleotides extending from position 227 of the satellite sequence through to the 3' nucleotide of the satellite unit at position 335 (TE 3'). Immediately 3' of this there is a complete satellite unit, in the same orientation as the incomplete unit, flanked at the 3' end by 16 nucleotides extending between the *Sma*I site and the *Eco*RI site of vector plasmid pT7-2 (ref. 22). The 105 construction is as 104, except that two complete satellite units, in the same orientation, are present. The insertions from 104 and 105 were excised by digestion with restriction endonucleases *Bam*HI and *Eco*RI, rendered blunt-ended by DNA polymerase (Klenow fragment) and inserted into the *Bam*HI site of binary Ti plasmid Rok1. The *Bam*HI site of Rok1 was rendered flush-ended by DNA polymerase (Klenow fragment). Rok1 is a derivative of the binary vector Bin19, described previously¹¹, containing additionally an expression cassette inserted into the polylinker site. The expression cassette comprised the promoter of cauliflower mosaic virus 35S RNA²³ and the terminator fragment of the nopaline synthase (*nos*) gene of *Agrobacterium* T-DNA²⁴ (NOS TERM). It is essentially similar to the expression cassette described for the expression of tobacco mosaic virus coat protein gene¹², except that the promoter fragment was deleted back to 6 bp 3' of the cap site of the 35S transcript using *Bal*31 nuclease. The unique *Bam*HI site was inserted between the promoter and terminator fragments. Transcripts derived from this cassette would contain 10 nucleotides from the promoter and 200 nucleotides from the terminator²⁴ in addition to the sequence inserted at the *Bam*HI site. The figure shows only the region adjacent to the cDNA insert of pT104 and pT105 and the expression cassette region of Rok1.

plants¹ or protoplasts⁴ that are infected with CMV. CMV isolates that do not contain satellite RNA can be cultured repeatedly in plants in a satellite-free state, but when satellite RNA is added to such cultures it is synthesized and persists as a component of the virus isolate⁵. The effect of satellite RNA on CMV infections depends on the strain of satellite: in many cases the usual symptoms of CMV are suppressed and as a result the infected plants show few symptoms of infection⁶⁻⁸. However, the presence of other strains of satellite RNA leads to the production of severe symptoms that are quite distinct from those of CMV⁸. Here we describe the transformation of tobacco plants with DNA copies of CMV satellite RNA, the production of satellite RNA transcripts by such plants and the acquisition of satellite RNA by CMV cultures grown in them. These results suggest a means of protecting plants against the effects of CMV and also suggest a method by which satellite RNAs may have evolved.

The satellite RNA used in this work was isolated from CMV strain I₇N (ref. 7) and was cloned as complementary DNA. The nucleotide sequence of the cDNA clone was very similar to that of other strains of CMV satellite^{2,9,10} and will be presented elsewhere, together with the construction of two head-to-tail concatemer satellite cDNA molecules. These comprised one (pT104) or two (pT105) complete satellite RNA sequence units

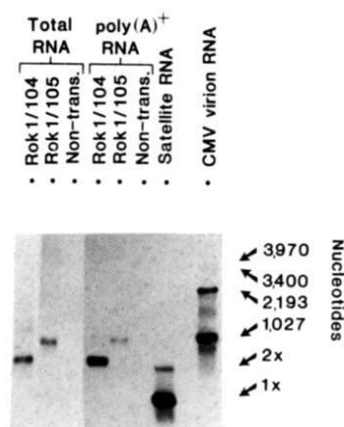


Fig. 2 Northern blot analysis of RNA from transformed plants. Total RNA (20 µg) or poly(A)⁺ RNA (1 µg) was isolated from leaves of non-transformed plants (non-trans.) or plants transformed with Rok1/104 and Rok1/105 and fractionated on formaldehyde/agarose gels. The transcribed satellite RNA sequences were detected by blotting the gel onto nitrocellulose and hybridization with nick-translated insert from pT105. Total RNA from cells infected with CMV which contained satellite RNA was included as a reference for satellite RNA unit length (1×) and dimer length (2×) molecules. The size markers were virion RNA of CMV, which was detected with a cDNA clone (C20) of CMV genomic RNA (D.C.B., unpublished). This hybridized with CMV RNA-3, CMV RNA-4 and faintly with CMV RNA-1 and RNA-2. The size values given are those of Gould and Symons²⁵.

Methods. The constructions of pT104 and pT105 in Rok1 were mobilized into *Agrobacterium* strain LBA 4404 using *Escherichia coli* strain HB101 harbouring pRK2013 in a triparental mating, as described previously¹¹. The resultant *Agrobacterium* strains containing the binary vector constructions were then used to infect leaf disks from axenically grown *N. tabacum* (cv. Samsun NN). The infected and transformed cells were regenerated into plants by culturing the disks successively on a layer of tobacco feeder cells and then on shoot- and root-inducing medium as described by Horsch *et al.*²⁶. The shoot- and root-induction media contained kanamycin at 100 µg ml⁻¹ to select for cells expressing the neomycin phosphotransferase gene contained in the T-DNA region of Rok1 (from Bin19)¹¹. Transformed plants were propagated by transfer of stem cuttings into root induction medium. RNA isolations were as described by Apel and Kloppstech²⁷ and electrophoresis and blotting analysis as described by Goldberg²⁸ and Thomas²⁹.

with incomplete units at the 5' end (Fig. 1) and were intended to mimic multimeric satellite RNAs that are thought to be replication intermediates (D.C.B., M.A.M., B.D.H. and G.R.S., unpublished results). To achieve expression of these satellite sequences in transformed plants, the cDNA molecules were transferred into an expression vector (Rok1) based on the binary Ti plasmid system of *Agrobacterium tumefaciens*^{11,12}. The resultant plasmids are referred to as Rok1/104 and Rok1/105.

Transformed tobacco plants (cv. Samsun NN) were regenerated from *Agrobacterium*-infected leaf disks and were assayed for the presence of the satellite sequence in DNA and RNA by Southern and Northern blot analysis, respectively. The Southern blot analysis showed that the DNA of transformed plants contained the equivalent of 1–3 copies of the satellite cDNA per haploid genome, and also that the cDNA sequence, the promoter site and the RNA termination site had not been rearranged or deleted. The Northern blot analysis (Fig. 2) showed transcripts of ~700 nucleotides (Rok1/104) or 1,000 nucleotides (Rok1/105), which are the approximate sizes predicted from the location of promoter and terminator sequences in the original constructions (Fig. 1) and allowing for the presence of a 3' polyadenylate tract. These RNA molecules were readily detected in total RNA or in polyadenylated RNA. No virus-like

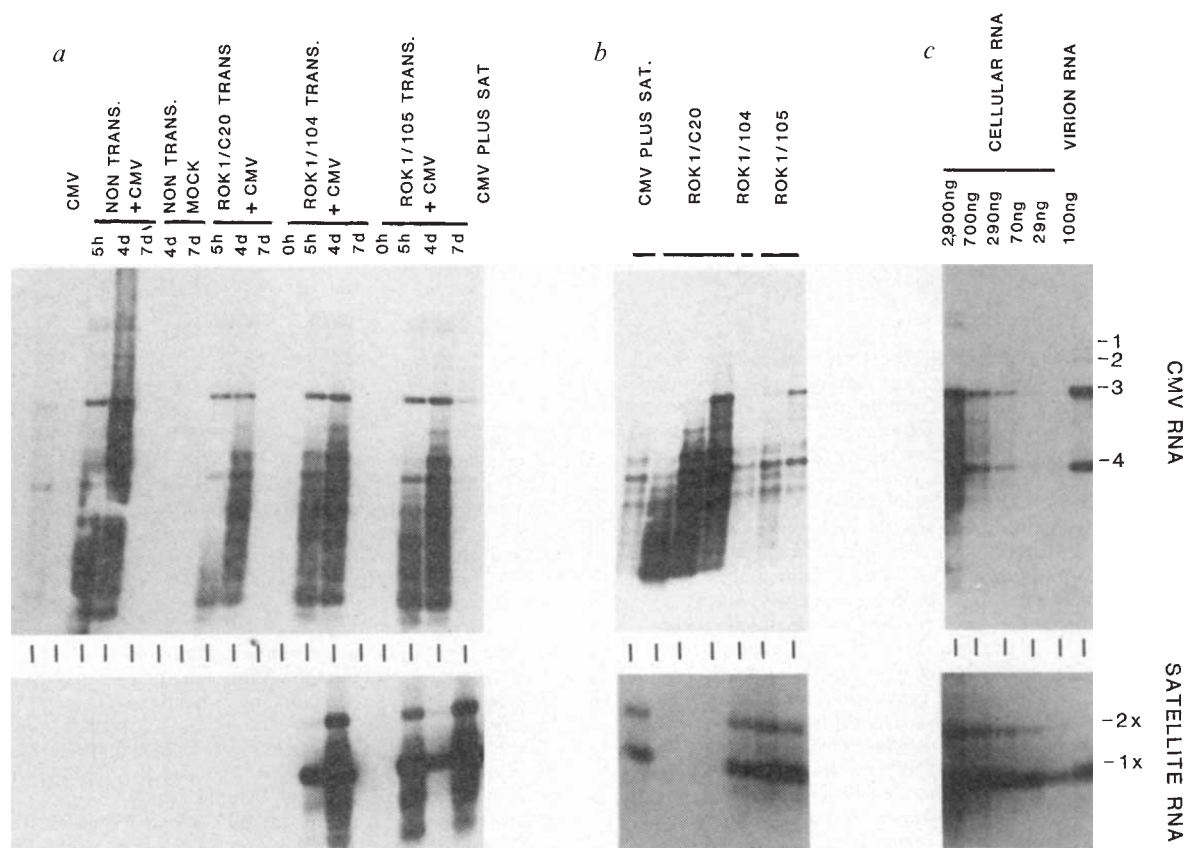


Fig. 3 Effect of CMV infection on the satellite RNA in transformed plants. Leaves of transformed or non-transformed *N. tabacum* (var. Samsun NN) were inoculated with CMV strain R76B and 35-mm disks were cut 5 h later. Batches of five disks were floated on SPS medium (10 g l^{-1} sucrose, $0.2 \text{ g l}^{-1} \text{ Ca} (\text{H}_2\text{PO}_4)_2 \cdot \text{H}_2\text{O}$, 0.3 g l^{-1} sulphanimide). Total RNA ($1 \mu\text{g}$) was isolated from these samples, or as indicated below, and analysed by Northern blotting as described for Fig. 2 using as probes the insert of pT105 (satellite RNA, lower panels) or C20 (CMV RNA, upper panels). The size markers were RNA of tissue infected with CMV alone or CMV plus satellite to show CMV RNA-1, CMV RNA-2, CMV RNA-3 and CMV RNA-4 or satellite monomer (1x) or dimer units (2x). **a**, RNA in primary infections. RNA was isolated from disks of non-transformed plants or from disks of plants transformed with the indicated derivatives of Rok1 at 5 h, 4 days or 7 days after inoculation with CMV R76B (+CMV) or buffer (mock). In some cases RNA was also isolated before inoculation (0 h). The Rok1/C20 construction was similar to Rok1/104 (Fig. 1) except that the insertion at the *Bam*HI site of Rok1 was a cDNA (C20) of the CMV coat protein mRNA. **b**, RNA in secondary infections. Extracts of CMV-infected leaf disks, similar to those described for **a**, were inoculated onto *N. clelandii* and RNA was isolated from the systemically infected tissue after 10 days. The RNA ($1 \mu\text{g}$) was isolated from plants inoculated with extracts of disks of plants transformed with Rok1/C20, Rok1/104 or Rok1/105. **c**, RNA in CMV particles. Disks from CMV (R76B)-infected plants transformed with Rok1/105 were extracted at 7 days after inoculation and the inoculum passed through non-transformed *N. clelandii* and re-inoculated onto *N. clelandii*. After 10 days of infection, CMV particles were isolated from systemically infected leaves³⁰ and the viral RNA extracted by addition of SDS to 1% and treatment with phenol. Total cellular RNA was also isolated from this tissue for comparison. The samples shown are 100 ng virion RNA or the indicated amounts of total cellular RNA.

symptoms or other abnormalities in growth, flowering or seed production were observed in the transformed plants.

To determine the biological activity of these transcribed RNA molecules, the transformed plants were infected with CMV strain R76B, a satellite-free isolate of CMV obtained originally from a naturally infected raspberry plant in Scotland¹³. In one such test, disks were cut from leaves 5 h after inoculation. RNA was isolated from the disks, either at that time, or after floating the disks on SPS medium for 4 or 7 days. Northern blot analysis of the RNA with a probe for CMV (C20) (Fig. 3a) showed that by 4 days the CMV genomic RNAs had accumulated in disks from both the transformed and the non-transformed plants. Many small RNA species which may represent subgenomic RNA species¹⁴ also accumulated over the same period (Fig. 3a). Satellite RNA was detected by Northern blot analysis after 4 and 7 days but only in the disks from plants transformed with Rok1/104 or Rok1/105 (Fig. 3a). This RNA was present in large amounts and was primarily of unit satellite RNA length although, as is normal for an infection containing satellite, some dimers were also detected (Fig. 3a). At the loading of RNA and

autoradiographic exposure of Fig. 3a, the transcribed satellite RNA molecules of ~700 and 1,000 nucleotides were not detectable. Similar results were obtained from analysis of inoculated leaves of intact plants.

Further tests showed that the satellite produced in transformed plants could be acquired as a permanent feature of the CMV culture. In one such test, sap from leaf disks in the experiments described above was inoculated to *Nicotiana clelandii* and RNA isolated from the systemically infected leaves after 10 days. The RNA was analysed by Northern blotting and found to contain satellite RNA in monomeric and dimeric forms, at levels typical of natural satellite RNA, when the inoculum had been obtained from disks of plants transformed with Rok1/104 or Rok1/105 (Fig. 3b). *N. clelandii* plants inoculated with CMV which had been passed through disks of Rok1/C20-transformed plants contained only CMV genomic RNA (Fig. 3b).

These experiments also showed that the production of symptoms in *N. clelandii* was markedly reduced when the inoculum contained satellite RNA acquired from transformed

plants. This was observed in all 12 plants inoculated with CMV R76B that had been passaged through plants transformed with Rok1/104 or Rok1/105 (M.A.M. and B.D.H., unpublished data). The effect of satellite RNA on symptom production could not be assayed in the actual transformed plants as CMV R76B produces very mild symptoms in *Nicotiana tabacum*.

The incorporation of satellite RNA from the transformed plants into virions of CMV was confirmed by Northern blot analysis of RNA isolated from CMV particles. These were isolated from a secondary infection of CMV R76B in *N. cleavelandii* following passage of the virus culture through Rok1/105-transformed plants (Fig. 3c). Both unit length and dimeric satellite RNA was detected, although there was less satellite RNA per unit of CMV RNA than in cellular RNA from the same plants (Fig. 3c). This effect was more marked with the dimer than the monomer satellite.

We also found that monomeric and dimeric satellite RNA accumulated to high levels in systemically infected leaves of CMV (R76B)-inoculated plants which had been transformed with Rok1/104 and Rok1/105 (D.C.B., G.R.S., M.A.M. and B.D.H., unpublished data). Satellite RNA was not detected in the systemically infected leaves of non-transformed or Rok1/C20-transformed plants. Further investigation will determine whether satellite produced in the transformed plants affects CMV replication and accumulation.

Clearly the transcribed satellite RNA in transformed plants can be processed to unit-length molecules following CMV infection and subsequently acquired and perpetuated as a new part of the virus culture. We believe this provides the first proof of the acquisition by a plant virus of genetic material from its host, a process that may be important in the evolution of plant viruses and their satellites. Indeed, although there is no evidence for the occurrence of CMV satellite-like sequences in DNA of non-infected tobacco plants¹⁵, sequences resembling those of the satellite RNA of turnip crinkle virus are reported to occur in the DNA of non-infected turnip plants¹⁶.

The fact that plants infected with CMV cultures containing a benign satellite RNA show milder symptoms than plants infected with satellite-free cultures⁸ suggests that satellite RNA might be used to protect crop plants against the effects of CMV infection. Preliminary experiments have shown that pre-infection with a CMV strain containing such a satellite RNA gave protection against CMV symptoms¹⁷ and led to increased yield of pepper plants¹⁸. It has also been shown that infection of tomato plants with CMV strain containing a benign satellite RNA results in protection against the effects of a CMV culture containing a virulent satellite RNA^{19,20}. However, the use of an avirulent virus culture as the prophylactic agent may cause several problems²¹. The protecting strain may itself have adverse effects on growth and yield or spread to other, more sensitive, plant species. It is also possible that the avirulent strain may mutate to virulence or interact with other viruses to cause severe symptoms. However, some of these difficulties may be eliminated by the strategy demonstrated here in which biologically active satellite RNA is maintained in the plant by transcription from the nuclear genome.

We thank Suzanne Mason for technical help and Dr M. Jacquemond (Montfavet, France) for providing strains of CMV. This work was supported in part by the Rockefeller Foundation.

Received 27 January; accepted 26 March 1986.

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Sequence-directed curvature of DNA

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DNAs from both prokaryotic¹⁻⁴ and eukaryotic⁵⁻⁸ organisms have yielded restriction fragments which manifest markedly anomalous electrophoretic behaviour (reduced mobility) when run on polyacrylamide gels. We have shown previously⁹ that the abnormal electrophoretic behaviour of one such fragment is a consequence of stable curvature of the helix axis in solution. The molecules involved tend to contain oligo(dA)-oligo(dT) runs which are approximately in-phase with the helix repeat^{7,8,10}; however, the precise structural elements responsible for DNA curvature have not been identified. One popular model^{11,12} for curvature invokes a non-coplanar 'wedge-like' conformation of ApA/TpT dinucleotide pairs. Despite a lack of direct evidence in support of this model, it has been used to provide quantitative estimates of curvature^{4,13,14}. To critically evaluate the ApA wedge model, we have performed an electrophoretic analysis of a series of closely related DNA polymers in which oligo(dA)-oligo(dT) runs of different polarity were compared. We conclude that ApA dinucleotide wedges cannot account for DNA curvature. Therefore, quantitative estimates for ApA wedge deformations, based solely on apparent curvature, cannot be correct.

DNA sequences displaying apparent curvature have been found at both the site of integration¹ and the origin of replication⁴ of bacteriophage λ as well as in regions of conserved sequence in trypanosomatid kinetoplast DNA⁷. Disruption of a region of curvature upstream from the histidine transfer RNA gene in *Salmonella* appears to be associated with down-regulation of tRNA^{His} transcription³. However, the functional significance of curvature *per se* remains obscure, as does its molecular origin.

Marini *et al.*¹² invoked the notion of non-coplanarity of ApA/TpT dinucleotide pairs, proposed earlier by Trifonov and Sussman¹¹, as a possible basis for the observed curvature. Other workers^{4,13,14} have attempted to provide a quantitative relationship between the hypothetical ApA wedge angle and the overall curvature of the helix axis. We have therefore attempted to test the hypothesis that an inherent non-coplanarity of ApA/TpT dinucleotide residues determines the contour of the helix axis in solution. In testing this hypothesis, one can invoke the following simple symmetry argument: for curvature arising solely from ApA wedges, the angle-of-transit of the helix axis through the sequence 5'-A_nT_n-3' (one strand shown) will be equivalent to the corresponding angle-of-transit through 5'-T_nA_n-3'. Should the angles-of-transit through these two sequences prove to be different, one cannot relate apparent curvatures (for example based on gel mobilities) to ApA dinucleotide non-coplanarity. We have shown recently¹⁰ that certain DNA oligomeric

Fig. 1 Demonstration of the anomalous electrophoretic behaviour of certain repeating-block copolymers of DNA. Lane *b* contains polymers of the form $[G_3TCGAC_3]_N$ where $2 < N < 25$. The electrophoretic mobilities of all members of this set are normal, *c*, $[G_2A_3T_3C_2]_N$; *d*, $[GA_4T_4C]_N$. The members of the latter two series show distinctly abnormal electrophoretic mobilities for $N > 5$. Lanes *a* and *e* contain DNA molecules derived from pBR322 by *Hae*III digestion; *f*, *Hpa*II digest of pBR322.

Methods. Representative fragment sizes are specified to the left of the gel. All gels used for this study were 12% polyacrylamide (37:1 monomer/bis) and were run at $24 \pm 2^\circ\text{C}$ (running buffer: 40 mM Tris-acetate, 20 mM sodium acetate, 1 mM Na-EDTA, pH 7.9). Polymers of varying N were constructed from synthetic decamers by phosphorylation and subsequent ligation (with T_4 DNA ligase in 0.5 mM ATP, 50 mM Tris-HCl, 10 mM $MgCl_2$ pH 8.0, at $12-15^\circ\text{C}$). Ligase concentrations and reaction times were varied in order to produce informative gels; the gel patterns do not represent maximum extents of polymerization. Ligation reactions were stopped by adding Na-EDTA to a final concentration of 20 mM, followed by extraction with phenol/ether. Oligodeoxynucleotides were synthesized manually using the phosphite triester chemistry of Caruthers^{15,16}. Oligomer sequences were verified by (1) using the chemical sequencing technique of Maxam and Gilbert¹⁷, with minor modifications for short oligomers, and (2) complete digestion of the polymerized decamers with the appropriate restriction endonucleases.

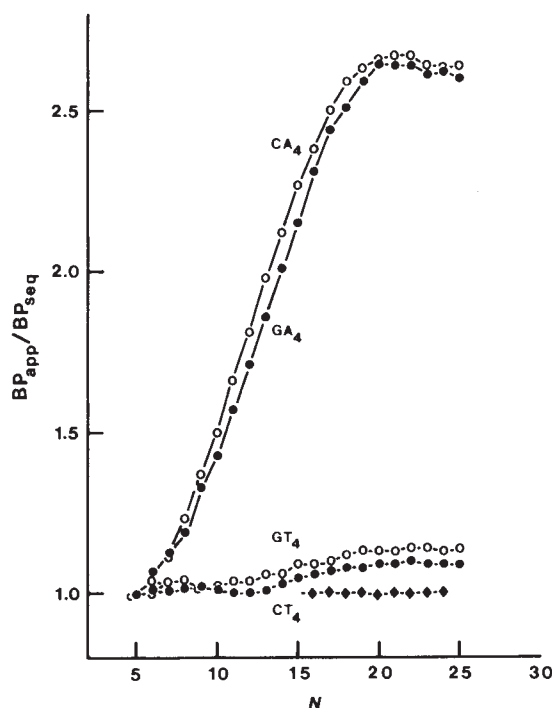
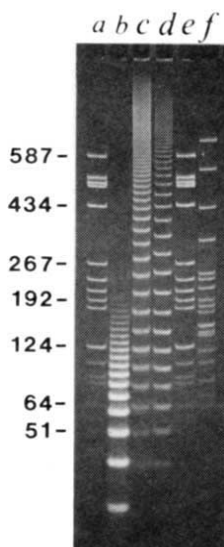


Fig. 2 Gel electrophoretic behaviours of duplex polymers having a repeating decamer motif. CA_4 , $[CA_4T_4G]_N$; GA_4 , $[GA_4T_4C]_N$; GT_4 , $[GT_4A_4C]_N$; CT_4 , $[CT_4A_4G]_N$. Mobilities of the various polymers, represented as the ratio of the apparent number of base pairs (BP_{app}) to the true number of base pairs (BP_{seq}), are plotted as a function of the degree of polymerization, N . The two curves plotted with solid circles represent sequence inversions of one another; the same applies to the two curves with open circles. ♦, $[G_3TCGAC_3]_N$ (lane *b* of Fig. 1, displaying a normal electrophoretic pattern for a decamer-based series).

sequences, when propagated approximately in-phase with the helix repeat, give rise to significant macroscopic curvature of the helix axis. That study exploited the fact that curved molecules migrate more slowly through polyacrylamide gels than do their linear counterparts (Fig. 1). Figure 2 compares the behaviour of the polymer series $5'-[GA_4T_4C]_N-3'$ with its counterpart $5'-[GT_4A_4C]_N-3'$. As the only difference between these polymers is the polarity of the A-T block, they would have to display identical electrophoretic behaviour if ApA/TpT dinucleotide wedges were determining the contour of the helix axis; clearly, this is not the case, as the $5'-T_4A_4-3'$ -containing polymers display nearly normal electrophoretic behaviour, in stark contrast to the $5'-A_4T_4-3'$ -containing species. This analysis does not rule out any participation of wedge-like deformations in generating curvature; however, such wedges, if present, do not determine curvature. Hence, it is incorrect to relate macroscopic curvature to an apparent ApA wedge angle. Note that the G and C residues flanking the A-T runs do not appear to influence the degree of curvature, since interchange of these residues (Fig. 2) has essentially no effect on the relative electrophoretic mobilities.

This research was supported by NIH grant GM28293.

Received 12 August 1985; accepted 20 March 1986.

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Errata

Danish Basin subsidence by Triassic rifting on a lithosphere cooling background

K. Sørensen

Nature **319**, 660-663 (1986)

ON page 662, Figures 2 and 3 have been transposed. The figure legends are correct.

An *Agrobacterium* transformation in the evolution of the genus *Nicotiana*

I. J. Furner *et al.*

Nature **319**, 422-427 (1986)

ON pages 424 and 425, the figures and their legends were labelled incorrectly: Figure 3 should be Figure 4 and vice versa.

Carbohydrate detection gains potential

from Dennis C. Johnson

Combined with liquid chromatography, pulsed amperometric detection extends the capabilities of carbohydrate analysis. The method allows direct electrochemical detection.

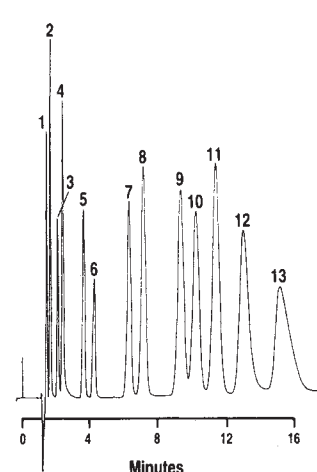
THE promise of analytical organic liquid chromatography (LC), enhanced over the past decade by improvements in hardware and methodology, has stimulated the search for new detection concepts to permit greater exploitation of LC capabilities. Detection based on UV-vis photometry (absorbance and fluorescence) has been most common in organic LC; however, photometric detection yields significant results only with unsaturated and aromatic molecules. For aliphatic analytes, photometric detection suffers from poor sensitivity unless aromatic functional groups are attached to the aliphatic molecules using tedious chemical derivatizations.

EC detection

Oxidative electrochemical (EC) detection has also proved useful for aromatic organic molecules such as phenols and biogenic amines and metabolites^{1,2}. In contrast, direct anodic detection of underivatized aliphatic organic molecules under constant (d.c.) applied electrode potentials is essentially unknown. Thermodynamic data predict oxidations in aqueous and mixed solvents. Hence, the apparent electroinactivity of aliphatic molecules at constant potential originates from kinetics problems, for instance the lack of resonance-stabilized free-radical reaction products.

Aliphatic molecules frequently generate transient anodic signals at noble metal electrodes, with the magnitude of the temporary signal and speed of its attenuation being a function of the electrode material. Because the electrode surface appears to interact with analyte molecules and/or reaction products, the reaction mechanisms are characterized as electrocatalytic³⁻⁵.

The example of aliphatic molecule electrochemistry that is best understood is the



Sugar	ppm
1. Inositol	20
2. Xylitol	20
3. Sorbitol	20
4. Mannitol	40
5. Fucose	50
6. Deoxyribose	100
7. Rhamnose	100
8. Arabinose	50
9. Galactose	50
10. Glucose	50
11. Xylose	100
12. Fructose	100
13. Sucrose	300

Fig.2 Separations of carbohydrates using anion-exchange chromatography with electrochemical detection. The column and detection are as in Fig.1; the mobile phase was 8.0 mM Ba(OH)₂/0.5 mM acetic acid. (Provided by W.T. Edwards, Dionex Corp.)

oxidation of simple alcohols at noble-metal electrodes³⁻⁵. The standard potential (E^0) for the conversion of methanol to CO₂ in aqueous solutions is predicted to be 0.0V versus NHE (normal hydrogen electrode). Electrode potentials many hundreds of millivolts more positive than this E^0 can be applied at common anode materials without causing decomposition of the aqueous solvent. It follows that all alcohols should be easily detected by electrochemical oxidation reactions.

However, the oxidations of alcohols are not 100 per cent efficient and free-radical intermediates are adsorbed strongly at the noble-metal anode surfaces, thereby eliminating further activity of those surface sites. Electrode currents for alcohols at noble metal anodes fall to zero within a few seconds in acidic or alkaline media³⁻⁵.

The adsorbed hydrocarbon materials which foul Pt and Au electrode surfaces can be eliminated by oxidative desorption when a large positive potential pulse is applied^{6,7}. A subsequent negative potential pulse cathodically dissolves surface oxides, restoring the full activity of the metal electrode surfaces.

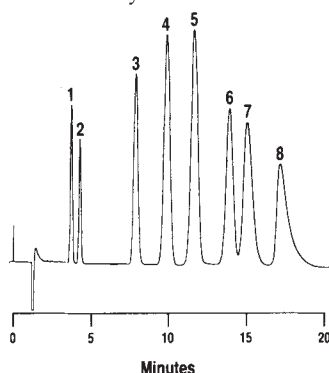
Cleaning pulses for analytical anodic potential are under the control of multi-step potential waveforms⁸. Each application of the detection potential, with measurement of electrode current, is followed by the positive and negative pulses for surface reactivation. Typically, the waveform is applied with a cycle frequency of about 1 Hz (1 cycle per second). The analytical technique has become known as pulsed amperometric detection (PAD).

PAD and chromatography

Response to PAD has been verified for all carbohydrates tested at Pt⁸⁻¹⁰ and Au^{11,12} electrodes: sugar alcohols (such as sorbitol), aldoses (glucose), ketoses (fructose), polysaccharides (sucrose) and aminosaccharides.

Because of the electrocatalytic basis of PAD for carbohydrates, the potential dependence of the anodic response is virtually independent of the identity of the molecule. The technique cannot distinguish between different types of carbohydrate in a mixture. Therefore PAD is applied as a general electrochemical detector for carbohydrates in the chromatographic effluent stream.

Among the possible designs for LC electrochemical flow-through detectors^{13,14} the most popular is the thin-layer cell. In this design, the fluid stream is confined to a narrow channel cut into a thin Teflon sheet (< 0.2 mm) separating two flat plastic plates (Kel-F). For PAD of carbohydrates, the indicating electrode (Au) and counter electrode (Au, Pt or carbon) are the flat end surfaces of rods (about 1 to 2 mm diameter) pressed into holes drilled



Sugar	ppm
1. Fucose	25
2. Deoxyribose	25
3. Arabinose	25
4. Galactose	25
5. Glucose	25
6. Xylose	25
7. Mannose	50
8. Fructose	50

Fig.1 Separation of common monosaccharides by anion-exchange chromatography with electrochemical detection. A Dionex HPIC AS-6 column with a 1.0 mM Ba(OH)₂/0.125 mM acetic acid mobile phase was used, followed by detection with a Dionex pulsed amperometric detector. (Provided by W.T. Edwards, Dionex Corp.)

in one plastic plate. The Ag-AgCl reference electrode, mounted in the opposite plate, is separated from the fluid stream by a cation-exchange membrane.

Any chromatographic system designed for carbohydrate LC-PAD must recognize the requirement for a supporting electrolyte and the desirability of maintaining a high pH to maximize sensitivity. It is preferable that the buffered supporting electrolyte serve as the chromatographic mobile phase. Low-capacity anion exchange columns, such as those used in ion chromatography¹⁵⁻¹⁷, have been successful in this application^{11,12,18}. Cation exchange columns in the Ca²⁺-form are also effective where pure H₂O is the mobile phase, provided an appropriate electrolyte/buffer solution is mixed with the column effluent stream prior to detection⁹.

Separation of a mixture of common monosaccharides is shown in Fig. 1; Fig. 2 shows the separation of a more complex mixture of carbohydrates. The retention mechanism apparently operates by ion exchange of the weakly ionized alcoholic groups under the conditions of moderately high pH. Sugar alcohols have low retention under these chromatographic conditions, and oligosaccharides are retained more strongly than the monosaccharides. Detection limits are approximately at the part-per-million (p.p.m.) level.

Analysis by LC-PAD has already been applied to fruit juices and alcoholic beverages^{9,18} and other food products¹⁵. In the future, clinical laboratories may use LC-PAD to obtain carbohydrate profiles of physiological specimens. Ultimately the analytical potential of LC-PAD may reveal the physiological function of many carbohydrates. □

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Analytica instrumentation

Munich comes alive this June with chemical technology to count, size and analyse.

HYBRITECH's new fully **automated immunoassay analyser** will be on display at Analytica. The Photon-Era can perform most of the manual laboratory procedures associated with Tandem-E immunoassay, including precisely-timed pipetting, aspirating, washing and quenching (Reader Service No. 100). The machine measures samples by way of a fixed- or discrete-wavelength spectrophotometer. Equipped with an IBM-PC XT system, Photo-Era's processing unit is comprised of two independent sections: the sample processor and the reaction processor. Together, these components can



Hybritech puts immunoassays on their own.

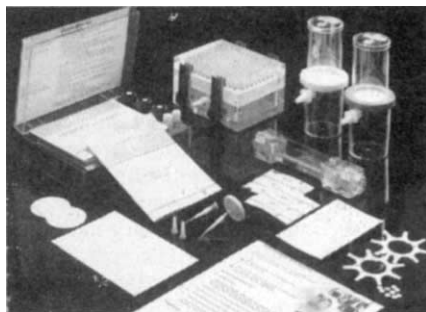
process up to 100 samples per hour, and can serve as general automated pipettor/diluters or spectrophotometer/spectrofluorimeters as well.

A **bench-top liquid scintillation counter** for bioresearch, biotechnology and clinical laboratories fills out Beckman's LS 5801 series. Beckman has designed the LS 1701 for quick and simple routine analyses (Reader Service No. 101). The standard instrument features three independently programmable user files, with preset isotope settings for tritium, carbon-14, phosphorous-32, sulphur-35 and iodine-125, as well as automatic isotope endpoint determination so that the system can adjust itself to any isotope selected. Beckman's machine can provide accurate d.p.m. data regardless of the scintillation cocktail or counting vials being used, and it retains its accuracy throughout changes in volume, quench types or isotope pairs and ratios.

Using protein pigments that occur naturally in algae and cyanobacteria, Biomedica Corp. has developed **immuno-fluorescent probes** that exhibit 10 to 30 times the fluorescent intensity of conventional fluorescein conjugates. Bound covalently to specific antibodies, these phycobiliproteins can combine recognition with fluorescence emission that is uniform over a wide pH range, and in the presence of most biological compounds (Reader Service No. 102). Biomedica's latest addition to their offerings, Phycopro-

be-PE (for phycoerythrin) antibodies, can be excited at the same wavelength as fluorescein-labelled compounds, although the Phycoprobe's maxima is at 575 nm. The higher wavelength transmission can translate into a clearer signal, because it creates a larger Stoke's shift.

Schleicher and Schuell, showing in hall 3 at stand B4, plan to present several new products at Analytica including an **electro-elution system** for the purification and concentration of electrically-charged biopolymers. Underlying the Biotrap's efficacy is a novel membrane which, when subjected to an electrical field, retains only charged molecules of molecular weights $\geq 5,000$ — without adsorbing them (Reader Service No. 103). With Bio-



Schleicher and Schuell's Analytica array.

trap, DNA/RNA and protein samples can be eluted from gels, concentrated by dialysis and separated by isoelectric methods. Disposable filter units, DNA purification chromatography columns and transfer media will also adorn Schleicher and Schuell's exhibit area.

Filter and ferment

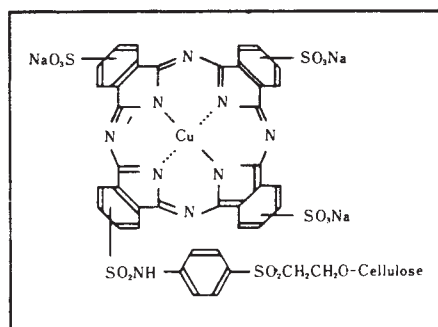
The star of Elga's show in hall 14 stand B16 is the Elgastat UHQ laboratory **water purification unit**. Incorporating five different purification techniques, the Elgastat unit yields high quality water for use in HPLC, fluorescence analysis, atomic absorption and ion chromatography (Reader Service No. 104). Water processed through Elga's system conforms to the following specifications: inorganics, 18 M Ω cm⁻¹ at 25°C; organics, 0.0001 absorbance at 254 nm; TOC, 20 parts per 10⁹; bacteria, 1 CFU ml⁻¹ and particles, 0.2 μ m absolute filtration.

In hall 2 stand B14/D, a variety of **fume filtration cupboards** from ASTEC Environmental Systems Ltd will grab the spotlight. All ASTEC's cupboards can be installed without ducting or building work, or mounted on trolleys for the

greatest flexibility (Reader Service No. 105). And 13 types of filters are available to combat the effects of solvents, acids, alkalis, ammonia, H_2S , SO_2 , formaldehyde, resins and so on. ASTEC's latest cupboard series, called Condor, has powder handling and cytotoxic drug preparation facilities and can be used as a glove box as well. It features high- and low-level extraction vents, a floor-level filtration system and a 1,200 mm $\times$ 600 mm epoxy resin dished work surface. Also exhibited in ASTEC's stand are the Astecair filtration fume cupboards and Mystaire scrubber.

A fermenter family from Infors AG will be making its world premier at Analytica this year, in hall 11 stand B15. Infors says it set out to build fermenters that were easier to handle, maintenance-free, more versatile and safer to operate (Reader Service No. 106). How well they have achieved their purposes can be judged first-hand. Compact ISF-fermenters can use top or bottom drive, selected at sight without tools or service engineers. The bottom valve, with high current flow, is combined with a sampling port which allows the extraction of very small probes. Infors guarantees sterilization *in situ*; when the sterilization procedure is initiated, the air inlet and outlet lines automatically separate from the vessel and can then be sterilized apart from the vessel. Peripheral measurement, together with control and regulation modules, complete Infors's ISF-fermenter package.

Aqueous and organic solutions can be cleansed of mutagens in minutes with a new adsorbent from Pierce, hall 14 stand



Mutasorb's formula for nabbing mutagens.

E17. Called Mutasorb, Pierce's agent consists of adsorbent cotton bearing covalently linked trisulpho-copper-phthalocyanine residues (Reader Service No. 107). This ligand enables Mutasorb to adsorb mutagens containing more than three fused aromatic rings, such as mutagenic polycyclic aromatic hydrocarbons, mutagenic heterocyclic amines, polycyclic antibiotics and dyes such as ethidium bromide and crystal violet. One gram of the adsorbent contains approximately 10 μ M of copper phthalocyanine; about 5 mg of Mutasorb per ml of solution can remove 1

μ M of ethidium bromide. Pierce's product can be added directly to aqueous solutions, whilst solid materials have to be extracted with water or organic solvents.

Next on the bench

Earlier this year, Sartorius brought a new line of analytical balances to the market with capacities up to 12,000 g, readabilities from 0.00001 g to 0.1 g and full-range electronic taring. Now at Analytica, Sartorius will show most of these balan-



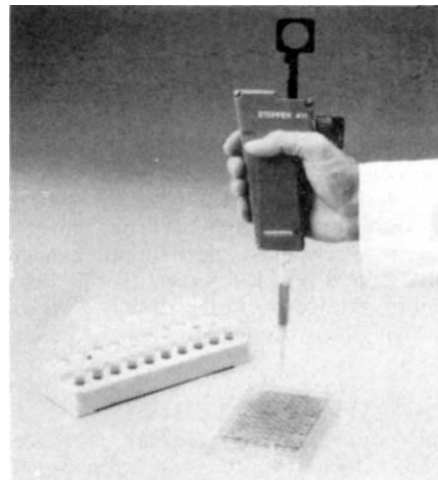
The A200S balance has clear advantages.

ces; and of these, the Analytic balance A200S may capture the most attention (Reader Service No. 108). Sartorius's top-loading A200S, with a 200 g capacity, features a weighing chamber housed in glass all around for 'five-sided' viewing. All balance functions can be controlled with up-front, one-finger formatting. One touch calibrates the balance, which then automatically returns to the weighing mode and displays sample weight. The Sartorius A200S has a 0.1-second update speed and an RS232C data output option that permits interfacing with printers, computers and Sartorius keyboards.

Carl Zeiss's research microscopes have just emerged from a design overhaul, and the results will be exhibited at Analytica. The Axioplan and Axiophot microscopes incorporate two design changes that should make switching between microscopic techniques more convenient (Reader Service No. 109). What Zeiss calls infinity colour-correlated system optics, or ICS optics, gives all objectives an infinite image distance so that a range of techniques including brightfield, dark-field, phase contrast, qualitative polarized light, differential interface contrast and fluorescence can be accommodated without additional lenses or loss of image quality. ICS optics also maintains a large consistent field of 25 mm and a contrast magnification factor, regardless of the method employed. System-integrated, or SI, design enables permanent or modular

optical components to be integrated into the microscope housing without impairing image quality or field of view. Components for either reflected or transmitted light microscopy fit the system equally well.

The adjustable volume repetitive dispenser lives up to its name: with this de-



Wheaton's dispenser: a cure for the common aliquot.

vice, up to 74 repetitive deliveries of pre-set microlitre volumes can be made without refilling the syringe. The dispenser is the brainchild of Wheaton Instruments (Reader Service No. 110). Filling the dispenser involves inserting a disposable syringe into the device's housing and raising the piston. A volume knob indicator can be rotated to the desired setting. Three sizes of disposable syringes effect total deliveries from 10 μ l to 5,000 μ l, with an accuracy of ± 1 per cent above 20 μ l, ± 1.5 per cent at 20 μ l and less.

As a primer for Ciba-Corning's Analytica exhibit, their 1986 laboratory instruments catalogue *World of Science* describes in full their Delta pH meters and ion analysers, their 410 digital flame photometers, carbon dioxide analysers, chloride analysers and colorimeters. The 20-page colour brochure gives individual specifications and information on accessories for Ciba's instruments (Reader Service No. 111). The catalogue also has lists of charts and brochures available on speci-



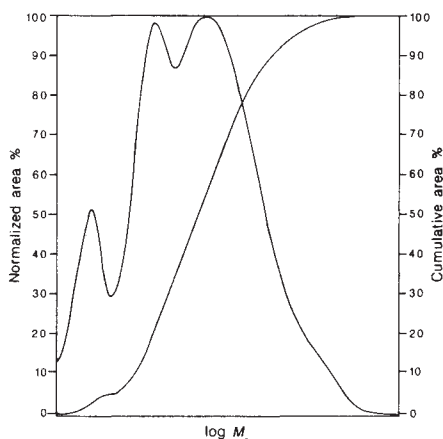
Essentials and accessories in Ciba-Corning's catalogue.

fic application methods should the reader wish to pursue a particular application in further detail.

Perkin-Elmer, in cooperation with the biotechnology company Cetus, have produced a **computerized pipetting system** specifically intended for biological procedures in microtitration assays. The Pro/Pette automated liquid handling instrument can be programmed, using a handheld controller, in 1- μ l increments throughout a 1- to 200- μ l range (Reader Service No. 112). With interchangeable pipette heads, Perkin-Elmer's device can perform serial dilutions, plate-to-plate transfers of samples, aspiration, filling and mixing of well contents. The ability to alternate between single- or multi-channel pipetting is another aspect of the Pro/Pette's flexibility. Perkin-Elmer plan to display their product in hall 5, stands A6/B3.

Software schemes

In conjunction with Heyden & Son's Chromatochart software, their new **gel permeation chromatography (GPC) software** for Apple II microcomputers can



Heyden MW distribution straight from the chart. perform molecular weight distribution calculations. Consisting of two modules, calibration and analysis, GPC enhance-

ment software is linked to Chromatochart's menu structure (Reader Service No. 113). The Software combines information from its parent's method, baseline and data files with calibration coefficients created by the calibration module. The GPC analysis module ties coefficient calculations together with Chromatochart's capabilities to correct for baseline drift and to define peak start and stop positions. Molecular weight distributions for the sample as a whole, or for individual peaks in a sample, can be obtained from Heyden's software package. Heyden can provide additional information on GPC software at their Analytica stand.

Two-dimensional gas chromatography, which can be built into any capillary GC system, just got its own microprocessing controller from Chrompack. At Analytica Chrompack will demonstrate the advantage of MUSIC, or multiple switching intelligent controller (Reader Service No. 114). MUSIC analysis begins by using a short 0.53 mm fused silica column to effect pre-separation of chromatography samples. Then, eluting peaks are trapped into a narrow-bore fused silica column with a different polarity, to achieve further separation. MUSIC's microprocessor allows fully automated operation and controls all flows, pressures, oven and trap temperatures.

Membranes, media and midgets

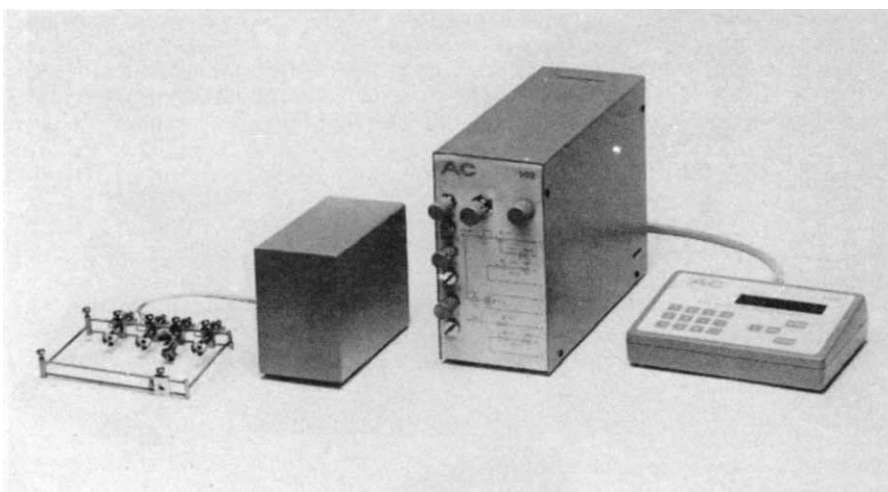
As an alternative to conventional cellulose dialysis sacs, Millipore is now offering V series membranes which can prepare microvolumes of proteins or nucleic acids for analysis via the **drop dialysis technique**. With this method, the V series membrane is floated on a Petri dish filled with dialysate, and the sample added to the membrane with a micropipette (Reader Service No. 115). The 0.05- or 0.025- μ m pore structure of Millipore's



Dialysis with a difference from Millipore membranes accelerates salt diffusion, and sample additions of 5 μ l to 100 μ l are probably less time-consuming than procedures used with cellulose bags. At Analytica Millipore can answer questions on their dialysis procedure. The company cites recovery rates in excess of 98 per cent with the drop dialysis technique.

Gold Label **dehydrated culture media** from BBL Microbiology Systems are the result of improved formulations that BBL says will provide superior test results. Currently BBL sell five varieties of the media (Reader Service No. 116). TSA II is BBL's improved general-purpose bacterial culture medium base for preparation with 5 per cent sheep blood. The critical components in another medium, Mueller Hinton II, are controlled to give consistent results in lot-to-lot susceptibility tests. BBL's third Gold Medal culture, MacConkey II, improves the inhibition of *Proteus* swarming so as to give other Gram-negative pathogens room to breath. GC II agar was developed in combination with BBL's IsoVitalEx enrichment and haemoglobin to facilitate the growth of *Neisseria gonorrhoeae* and other fastidious microorganisms. Finally, BBL has a grade A agar that, used as an ingredient, may improve growth and test reaction results.

In the race to catch up with Pharmacia's Phastsystem, LKB has introduced a **miniature electrophoresis system** that completes an analysis from gel-casting to scanning in under two hours. On display at Analytica, LKB's 2050 Midget system can perform screening of small samples by high-resolution techniques such as zone, disc, gradient, SDS and 2D electrophoresis (Reader Service No. 117). The complete Midget package includes multiple gel casting units, an electrophoresis tank, an electrophoretic transfer unit, a laser densitometer, digital power supply and accessories. The system can cast up to 14 gels at one time and run two gels with 5, 10 or 15 samples each in less than 45 minutes;



Chrompack will bring MUSIC to chromatography at this year's exhibit.

LKB claims the time savings can cut running costs by 90 per cent.

Newcomers in instruments...

Automation has won over Camag's Linomat spray-on techniques for **narrow-band sample application** in thin-layer chroma-



Camag sample preparation narrows down to TLC

tography. The Linomat IV is a microprocessor-controlled, programmable instrument with keyboard parameter entry that automates the same spray-on technique, using nitrogen as the carrier gas, that previously bore the Linomat title (Reader Service No. 118). Camag's latest version, exhibited in stand C8/D7, hall 2, computes the number of available tracks from information on band length, distance between samples, edge space and plate width. Linomat IV then indicates the tracks by means of light-emitting diodes, and sample deliveries from the syringe proceed automatically.

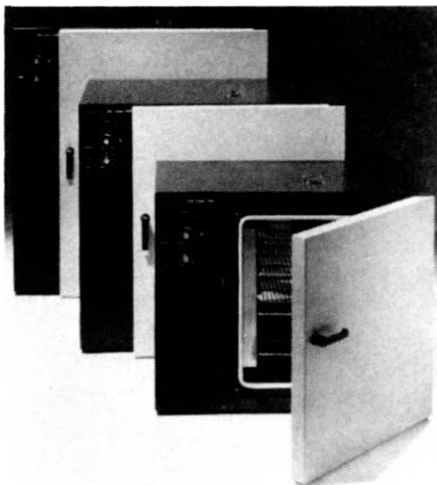
A **stopped-flow spectrophotometer** from Hi-Tech Scientific can generate five runs from 500 μ l of each reagent. Hi-Tech intended their instrument for work with precious biological materials, and minimized the quantities required to fill the flow circuit, which is totally thermostatted, chemically inert and readily aerobized (Reader Service No. 119). Their spectrophotometer features a two-tier design consisting of an upper sample handling platform and lower optical platform. The optical cell itself uses an integral ball-mixer and has four optical windows for 2- and 10-mm absorbance and fluorescence



LKB's Midget runs high-resolution separations in less than an hour.

work. At Analytica, Hi-Tech will be located in stand B14, hall 7.

The display at Gallenkamp's stand B3 hall 9 will reveal a rounded-out collection



Gallenkamp ovens in all shapes and sizes.

of **laboratory and light industrial ovens**, from standard 23-litre units to large 586-litre units, and from vacuum to humidity oven environments. Gallenkamp's most recent arrivals are the standard ovens sized up to 200 litres, in gravity-convected and fan-circulated versions (Reader Service No. 120). General-purpose ovens, which specialize in temperature control, come in 90- and 150-litre sizes. With a

temperature range of 40°C to 240°C, Gallenkamp's large-capacity ovens (340 and 586 litres) were designed for large loads or awkwardly-shaped items. And two large-capacity vacuum ovens in 90- and 260-litre volumes have joined Gallenkamp's 30-litre vacuum variety. The company will also have their incubator line, including the Gallenkamp Orbital models, at Analytica this week.

Bent on expanding the possible system configurations of their LC instruments, Spectra-Physics has added a **scanning UV-vis detector** to its product list. The SP8480XR connects with the Spectra-Physics computing integrators, SP4270 or SP4290, via a plug-in PROM chip installed by the user (Reader Service No. 121). The SCAN chip enables the integrator to perform automatic stop-flow peak scanning with background subtraction, peak ratioing and time-programmed wavelength

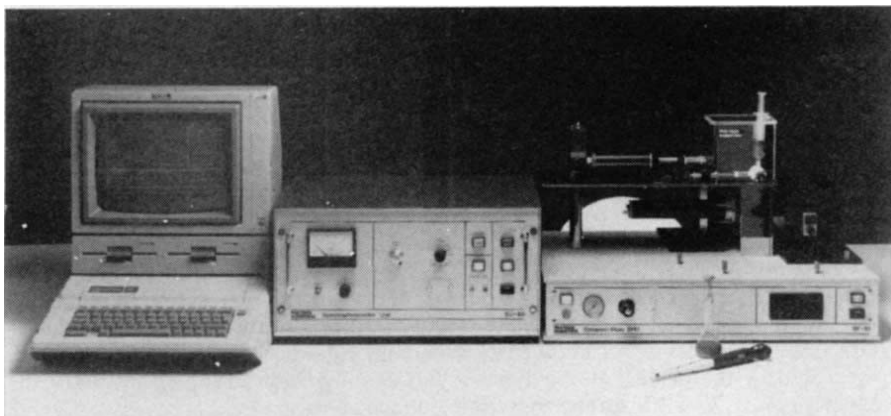


A UV/vis detector joins Spectra-Physics' LC outfit.

changes for selected peaks within a chromatogram. The detector itself has a drift measured at less than the 1/10,000th of an absorbance unit per hour. Baseline zeroing can be carried out manually or automatically. Spectra-Physics will have the scanning detector on display at their Analytica stand.

...and accessories

A **wideline solids accessory (WSA)** for use in biophysical research can expand the capabilities of Varian's VXR series of NMR spectrometers to include the examination of samples in solid states. With

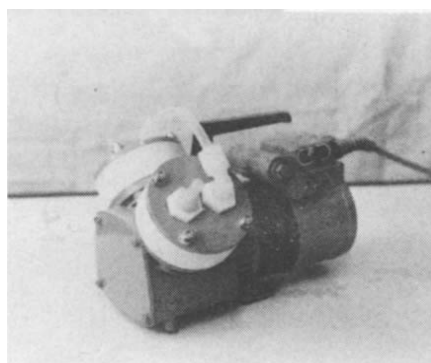


A stopped-flow spectrophotometer from Hi-Tech gets the most out of small samples.

Varian's new solids NMR instrumentation, researchers can obtain detailed structural and dynamic information about molecules and investigate molecular motion (Reader Service No. 122). WSA has a 1 kW high-power pulse amplifier that generates the short radio-frequency pulses needed to cover the wide spectral widths associated with solid protein NMR spectra. Likewise, the WSA receiver bandwidth has been increased to 2 MHz. Varian can also demonstrate the software included in WSA that performs specific wide-line NMR experiments on Varian's spectrometers.

A **sealed rotor** for middle-capacity benchtop and under-bench refrigerated centrifuges helps contain blood samples and other infectious materials during centrifugation. Heraeus Separationstechnik has engineered the 3360 rotor with a stainless steel shield and transparent polycarbonate lid to replace their 3350 rotor (Reader Service No. 123). The tightly-closing lid keeps infectious agents from getting into the centrifuge or surrounding areas in the event of aerosol formation or glass breakage, and breakage can be identified immediately because the lid is clear. Heraeus Separationstechnik's rotor also uses a racking system which caters to tubes of capacities from 7 to 100 ml, and has decanting and incubation facilities. More information will be available at Analytica from Heraeus Separationstechnik.

KNF Neuberger have machined a **vacuum pump head** from solid Teflon, resulting in a pump with strong chemical resistance to corrosive vapours. The inlet and outlet valves in KNF's pump are also made from Teflon, and the diaphragm is produced from rubber laminated to a thick layer of Teflon (Reader Service No.



A Teflon pump head cuts corrosion.

124). Teflon protection means that the pumps can be used with all kinds of chemical vapours without inevitable pump failure. KNF's series of corrosion-resistant pumps spans flow rates from 18 to 32 litres per minute, with vacuum levels down to 10 mbar.

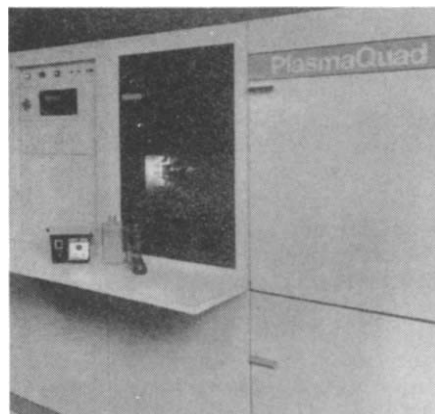
Savant Instruments, in stand D4, hall 7, take another approach to vapour corrosion in vacuum pumps. Their **chemical trap**, the model SCT120, removes acidic, alkali, organic and inorganic vapours that can condense in the pump system and wreak corrosive havoc (Reader Service No. 125). Savant's plexiglass cylinder measures 5 inches in diameter by 11 inches in height, with a removeable top for cleaning and re-charging. In operation, the SCT120 should be placed between the



Savant traps vapours before damage begins. concentrator/evaporator and the vacuum pump; standard 1/2-inch tubing will make adequate connections. □

A computer-assisted **data acquisition and analysis** system for VG Isotope's VG 9000 glow discharge mass spectrometer (GDMS) will make its debut at VG Isotopes' stand A10, hall 11 at Analytica (Reader Service No. 126). The system offers multipaging of screen memory for

access to menu-driven utilities such as current status readouts, real-time data acquisition displays, peak information and graphic analysis of old or current data. Graph scaling, peak identification and selection, baseline setting and other parameters are controlled from soft keys. VG Isotopes claim this package enhances the performance and convenience of their VG 9000, a GDMS instrument which has achieved detection limits below 10 parts per 10⁹. Also at the company's Analytica stand, VG Isotopes scientists will be operating the PlasmaQuad inductively coupled



VG Isotopes will be giving demonstrations of their ICP-MS at Analytica.

mass spectrometry system (ICP-MS), a machine that gives detection limits better than 0.1 parts per 10⁹ for most elements.

Baird & Tatlock's motorless stirring technique has some new followers. The Flatspin 2 range of **stirrers**, on view in Analytica at stand B3 hall 9, has a 'spin-off' feature that provides a warning if the magnetic follower loses capture while stirring (Reader Service No. 127). Baird & Tatlock have developed two types of Flatspin 2 products; the new immersion stirrer and a hotplate stirrer. The immersion stirrer has an encapsulated head that can withstand temperatures up to 100°C; the hotplate stirrer now includes an external probe for monitoring temperature suspended in solution. □

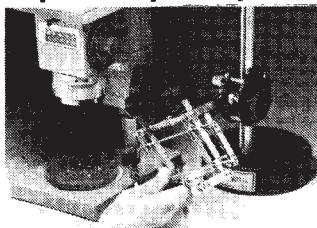
These notes are compiled by Karen Wright from information provided by the manufacturers. To obtain further details about these products, use the reader service card bound inside the journal.

Erratum: VCA/AIDS ELISA test kit

THE product review for Cellular Products' Retro-Tek AIDS test, published in the 10 April 1986 issue of *Nature*, incorrectly stated that "direct absorbance readings [generated by the test kit]... indicate the progress of the disease". In fact, these readings can only be used to monitor the concentration of the AIDS viral antigens in tissue culture samples or the presence of AIDS antigens in isolation and purification schemes. □

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